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"SWAYBACK" STUDIES IN NORTH DERBYSHIRE.

I.—THE FEEDING OF COPPER TO PREGNANT EWES IN THE CONTROL OF "SWAYBACK."

By G. DUNLOP,
(*County Agricultural Institute, Derby*),

J. R. M. INNES and G. D. SHEARER,
(*Institute of Animal Pathology, Cambridge*),

H. E. WELLS
(*Assist. Agricultural Organiser, N. Derbyshire*).

INTRODUCTION.

THE possible role of copper in the ætiology of "Swayback" was first suggested by Bennetts and Chapman (1937) in Australia. Their results and conclusions have already been discussed and are important as they suggested a new approach to the problem of demyelination. This was the first indication that a trace element might be vitally involved with myelin metabolism; because of the obscurity which cloaks the ætiology of the demyelination diseases this idea if confirmed might have far reaching results. Subsequent to Bennett's work Dunlop and Wells (1938) conducted a large field experiment to test the prophylactic value of Cu and to determine whether other trace elements (known to be deficient in this area) possessed prophylactic value. It was shown that when ewes on "affected" land had access to copper mineral licks for the last four months of gestation, the incidence of "Swayback" in their lambs was negligible.

In the autumn of 1938 it was considered that a controlled experiment carried out on a large scale would be of value in confirming and extending previous work. Such an experiment would also give scope for the more detailed chemical and hæmatological work which were a necessary corollary of any further research programme. It is the purpose of these papers to describe these experiments and the results.

Experimental Methods.

The investigation was made on Bradwell Moor which is the geographical centre of a large area in North Derbyshire where the incidence of " Swayback " has been high for many years. The farmers within this area were invited to co-operate in the work and a committee was formed to take over the administrative and financial side of the investigations. Forty-four farmers contributed 10 to 15 per cent. of their breeding stock and 260 ewes were thus obtained for experimental purposes. These ewes had either produced " Swayback " progeny in previous years or had been on this affected area for at least a year. Three hundred acres of good but " affected " land on the moor were placed at our disposal and the boundary stone walls were made sheep proof to divide the area into four separate but contiguous enclosures. A fifth enclosure, about a mile away from the main area, was taken over as an additional control field.

After their arrival on the experimental ground at the end of November, 1938, the 260 ewes were branded and numbered with ear tags for identification purposes. Mating had already begun early in November and the rams remained with the ewes on the experimental ground for some time. The experimental flock was then divided into the following groups, care being taken that each farmer's contribution of ewes was randomised over these groups as far as possible :—

Group.		*Treatment.*
A. Control group.	51 ewes.	No licks ; kept away from main area.
B. „ „	50 „	Pure salt licks only. No Cu.
C. Experimental.	55 „	Licks containing 1 per cent. Cu.
D. „	104 „	Licks, containing 0·3 per cent. Cu.

From December 9th, 1938, Group B and all the other animals in the experimental groups (C and D) before being drafted on to the copper licks, had access to licks containing only the purified salt. The above amounts of copper (as $CuSO_4$) were mixed with purified NaCl and make up into licks weighing 5 lb., one lick to 20 ewes. Subsequently a number of ewes from the experimental groups were drafted on to the copper lick treatment (0·3 and 1 per cent. Cu), on December 23rd, 1938, January 11th, 1939, February 1st, 1939 and February 23rd, 1939 or roughly for twelve, ten, seven and four weeks respectively before lambing. These groups are all shown along with the results in Table I.

The experimental groups C and D and control group B were moved around the contiguous enclosures in sequence every three weeks to ensure that all the ewes had access to the same herbage throughout the experiment. The appropriate mineral licks for each group were sheltered from weathering in covered hay racks and these were moved around the enclosures with the ewes. The ewes in control group A

were kept in the isolated field until just before lambing, when they were transferred to the main experimental area.

On December 20th, 1938, a heavy snowstorm occurred and the experimental ground, which lay at an altitude of 1,000 to 1,300 feet remained covered until mid-January. The herbage was also covered with snow for long periods in February and again in March. Supplementary feeding was thus essential ; the rations and amounts fed in lbs. per ewe were as follows :—

	Dec.	*Jan.*	*Feb.*	*Mar.*	*April.*	*Totals.*
Hay..	9	40	40	28	26	243
Concentrates	2	4·8	10	14·5	16·3	47·6
Sugar beet pulp	—	—	—	0·8	6	6·8

The hay was obtained from two badly " affected " farms ; the concentrates consisted of kibbled oats, wheat bran, flaked maize and ground nut flake. The Cu content of the mixed rations used during the experiment varied from 6·5 to 10·5 (p.p.m.).

Autopsies were made on all ewes which died during the course of the experiment. During the lambing period all lambing dates were recorded daily and the lambs were examined soon after birth. Some of the lambs severely affected with " Swayback " were killed, while others died. All lambs which died or were killed were subjected to a *post-mortem* examination, particular attention being paid to the nervous system for diagnosis. In those cases which showed macroscopic lesions further examination was obviously unnecessary. In the case of every other dead lamb, in which no gross lesions were present in the brain, portions of the brain and/or spinal cord were fixed in formalin-saline for subsequent histological work ; included in this series were all stillborn lambs and near-full-time aborted lambs. As some lambs were found dead without having been seen alive, this latter procedure was necessary to establish either a positive or negative diagnosis of " Swayback." The difficulties which attend the clinical diagnosis of the mild cases of " Swayback " are great as the slight staggering associated with posterior weakness seen in such cases might be simulated by a number of different conditions. For the purposes of our records all such animals were regarded as cases of " Swayback " ; a few were killed and the cord or brain examined histologically to establish a diagnosis. Some of these cases did not show lesions of " Swayback." We did not include in this category lambs which showed slight symptoms a month to six weeks after being returned to the respective owners, as the lambs must then be regarded as being in a different environment.

This paper is entirely concerned with the effect of Cu licks on the incidence of " Swayback " in the progeny of ewes in the experiment. The haematological and chemical work which was done on the ewes at different times during pregnancy will be dealt with in a subsequent communication.

Results.

Lambing began on March 13th ; for the first ten days bad weather was experienced and there were many stillborn lambs and deaths shortly after birth. As lambing progressed, however, the mortality rate decreased and after mid-April almost all normal lambs were successfully reared. It has been suggested by some farmers that "Swayback" occurs more frequently in the lambs born at the beginning of lambing. There is no correlation between the occurrence of "Swayback" and the total mortality wi h any particular time of the lambing period.

The results of feeding copper at different levels in mineral licks to pregnant ewes over various periods before parturition are summarised in tabular form (Table I). These are self-explanatory and require little comment or discussion.

Table I

Number of Lambing Ewes, Births, Deaths and Cases of "Swayback" in the various Groups

Group.	*No. * of Ewes.*	*No. of Lambs Born.*	*No. of Lambs Died.*	*† Cases of "Swayback."*	*‡ Deaths from all other Causes.*	*No. of Ewes with Swayback Lambs.*
Control A ...	40	63	40	24[1]	18	16
Control B ...	40	60	28	12[2]	17	10
Total ...	80	123	68	36	35	26
Experimental C. 1 per cent. Cu from—						
December 23	15	27	7	0	7	0
January 11	10	16	4	0	4	0
February 1	11	17	5	1	4	1
February 23	10	17	4	1	3	1
Total ...	46	77	20	2	18	2
Experimental D. 0·3 per cent. Cu from—						
December 23	32	50	20	0	20	0
January 11	15	20	3	1	2	1
February 1	16	25	7	4	3	3
February 23	18	28	13	8[3]	6	6
Total ...	81	123	43	13	31	10

*The numbers in this column are less than the original number placed in these groups at the beginning of the experiment owing to deaths, barren ewes, and animals which were missing at the final identification. There was no evidence whatsoever that the mortality in the ewes was due to the copper treatment ; the causes of death included obvious infection, pneumonia, one case of cirrhosis and deaths during parturition from obstetrical troubles.

†The cases of "Swayback" include a few animals which showed symptoms shortly after birth and were not killed. [1]Two cases, [2]One case, [3]One case.

‡This includes stillborn lambs.

Two methods may be employed in analysing the results of this experiment: (1) the number of " Swayback " lambs as a percentage of total lambs born in any one group, or (2) the percentage of ewes which gave birth to affected progeny (whether singles, twins or triplets). As the disease is primarily one of intra-uterine origin and as it was the ewes which received the various treatments, the most logical method might be the latter. The results show, however, remarkably little difference whichever way is adopted.

It will be seen from the table that the incidence of " Swayback " is highest in the control group of ewes (A) which had been wintered in the enclosure away from the main experimental area; there were 24 cases of " Swayback " (two with symptoms only and not killed) out of 63 lambs born from 40 ewes, while in the other control group (B) there were twelve cases (one with symptoms only) from 60 lambs born from 40 ewes. Compared with these figures the *total* absence of the disease in the experimental groups (C and D) which had received Cu licks from December 23rd, 1938, until lambing (77 lambs from 47 ewes) is striking and convincing proof of the efficiency of copper in the prevention of " Swayback." It is also apparent that the incidence increased in the experimental groups accordingly as copper treatment was delayed; thus in the group which had received the Cu licks (0·3 per cent.) for only a short time before lambing (from February 23rd) there is little difference in the incidence compared with control group B. There is also a higher incidence of the disease in the groups which received 0·3 per cent. Cu licks compared with those which received 1 per cent. Cu licks from any of the given dates. The numbers of ewes in the various sub-groups appear to be too few to permit any conclusions being drawn regarding the critical period in gestation for copper treatment.

TABLE II

LAMBING RECORDS EXPRESSED IN PERCENTAGES; THE CASES OF " SWAYBACK " INCLUDE A FEW ANIMALS WHICH SHOWED SLIGHT SYMPTOMS AFTER BIRTH AND WERE NOT KILLED

Group.	*Per cent. Lamb Crop.*	*Total per cent. of Deaths.*	*Per cent. of Lambs with " Swayback."*	*Per cent. Deaths from all other Causes.*	*Per cent. of Ewes giving Birth to " Swayback " Lambs.*
Control A ...	157·5	63·5	38·1	28·6	40·0
Control B ...	150·0	46·7	20·0	28·3	30·0
Experimental D. (0·3 per cent. Cu)	151·9	34·9	10·5	25·2	12·3
Experimental C. (1 per cent. Cu)	167·3	25·8	2·5	23·4	4·4

Expressed in the form of percentages the differences between the control and experimental groups are even more striking. These are shown in Table II, in which the percentages of total deaths, cases of " Swayback " and deaths from all other causes are related to percentage lamb crop.

This table shows that the great increase in mortality of lambs in the control groups compared with the experimental groups is almost entirely due to " Swayback " or, in other words, that the death rate from all causes apart from " Swayback " is similar in all groups.

Conclusions.

From these results it is evident that the administration of copper contained in salt licks to pregnant ewes has a remarkable beneficial effect on the prevention of the disease in the progeny. It also appears from the extremely low incidence of the disease in the groups which had access to licks containing 1 per cent. Cu (two cases from 77 lambs) that satisfactory results might be equally obtained by increasing the amount of Cu and giving the treatment relatively late in pregnancy. The occurrence of only one case out of 17 lambs in the sub-group which received the Cu treatment only four weeks before lambing supports the conclusion derived from the pathological data namely that the demyelination process in " Swayback " does not begin in the fœtal lamb until a relatively late stage of intra-uterine life. If this were not so a higher percentage of cases would be expected to occur in these latter groups.

The Cu requirements of the ewe as far as the normal fœtal development is concerned must thus be maximal, or limited to the fœtal period when we know that cerebral myelination in the lamb begins to occur with any degree of activity, *i.e.*, about 15 to 16 weeks gestation.

These findings are highly important and confirm further the suggestions of Bennetts and Chapman, although it is fully realised that we cannot offer at this stage any explanation of the mode of action of this trace element or of the pathogenesis of the disease. Cu appears to be a vital factor in normal myelin metabolism but how, or in what manner it acts, is completely unknown.

As has been shown by Innes and Shearer (1939), there was ample evidence that the disease in the lamb is not one of a Cu deficiency *per se* in the pregnant ewe. While this paper presents evidence of a method for prevention of the disease, any great understanding of the ætiology must await the solution of various biochemical problems connected with the physiological role of copper and its relation to myelin metabolism in the animal organism.

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Literature.

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THE CONTROL OF CHRONIC STREPTOCOCCUS MASTITIS

By F. C. Minett,
Research Institute in Animal Pathology,
Royal Veterinary College, London

In this paper, an account is given of a small self-contained herd lightly infected with mastitis in which over a period of two years a series of examinations has been made of the milk of individual cows. In view of the very favourable conditions existing in this herd, one of the first objects was to see whether, after removal of the few cows with easily demonstrable latent infection, there would be left a group of animals free from the disease.

In 1933 Minett, Stableforth and Edwards showed that it was possible to eradicate chronic streptococcus mastitis from a herd by removing infected animals. At the time of their report the animals passed as clean and their progeny had been free from the disease for three and a half years and (private communication from Dr. Stableforth) tests at six-monthly intervals since have shown that the herd has remained free for nearly ten years. Stableforth, Edwards and Minett (1935), reporting on six other herds, found that in five of them, although the disease did not extend and new infections tended to become fewer, the disease was not eliminated. In the sixth herd, although the initial incidence reached 50 per cent., the disease was eradicated, due largely to early sale of infected animals. Emphasis was laid on two points, firstly that in a number of cows infections with *Str. agalactiae* were light and could only be revealed by enrichment methods and often disappeared spontaneously, and secondly that in attempts at control there was advantage in keeping newly calved heifers segregated from the older cows.

Particular reference should be made to the outstanding work of Steck, carried out in Switzerland from 1929 to 1939, on the control of mastitis. In the Canton of Bern a voluntary co-operative plan of control was inaugurated in 1929, and several reports of progress have been published since then. In 1938, a report from Professor Steck's laboratory indicated that out of 61 herds observed for periods up to about two years, 54 were regarded as efficiently controlled. Of these 54 herds, 39 had finally no infected cows, or practically none and 14 had greatly improved. The control measures in use consisted of eliminating certain infected animals and treating others with an acridine preparation known as " zysternal." The subjects of diagnosis, control and pathogenesis are also dealt with by Steck in articles published from 1932 to 1939. As early as 1932 Steck showed that there can exist a " cultural latency," where an udder quarter is infected, although examination of milk by methods capable of revealing as few as 2 to 10 streptococci per c.c. may be negative for periods as long as six months. The presence of such infections is known by the fact that when larger amounts of milk than say 0·5 or 1·0 c.c. milk are cultured it may be possible to demonstrate streptococci. Also, in the routine examination of udder milk samples very mild infections may be found, *i.e.*, where less than 20 streptococcus colonies develop from 1·0 c.c. fore milk. Such mild infections often disappear spontaneously, or they may occasionally develop into severe or moderately

severe infections. Owing to " cultural latency " and the existence of these mild infections, he believes that there is in practice little point in trying to distinguish between " mastitis-free " and " mastitis-infected " herds. In practice it would be more useful to speak of herds " relatively free " from mastitis and of " mastitis-infected " herds. By the latter would be understood herds where clinical cases of mastitis occur and where one or several animals permanently excrete streptococci in such numbers that more than 20 colonies develop from 1·0 c.c. of fore milk. In " relatively free " herds the disease only occasionally becomes obvious and with careful supervision there is never an outbreak, so that the disease remains agriculturally unimportant. Although little can be said as to the ultimate fate of these " relatively free " herds, Steck thinks that with careful watching they might gradually become entirely free from infection. In a badly infected herd, on the other hand, events may be quite different. Klimmer and Haupt (1938) give an account of one such herd, in which at the beginning 36 out of 51 milking cows were infected. Of the 15 cows which were free from infection 11 were retained in the herd and all these eventually became infected. During the four-year observation period, 51 animals, 49 of them heifers mostly born on the farm, were added and 60 animals were sold. Of the 49 heifers 24 were found to be infected during the first lactation, 8 during the second and 1 in the third ; of the remaining 16, 14 were known to have remained free during the first lactation and two during two lactations. Klimmer and Haupt also noted the spontaneous disappearance of infection in 13 cows, while there were also many cases where infection disappeared from individual quarters. They recognise three stages of infection, the first where the streptococci are commensal inhabitants of the gland and are present in small numbers only, the second stage marks the transfer from commensalism to parasitism and is accompanied by a greater multiplication of the organisms, and the third stage where the udder definitely reacts to infection. They point out that a study is required of the conditions which govern the progress of infection from one stage to another.

Herd BB.

This was the herd used by Wilson, Minett and Carling (1937) in studying the nutritive value of raw and of pasteurised milk for calf-rearing. The history of the herd is given in their article but for convenience the following details are repeated. The herd, which is made up on an average of 20 milking shorthorns, has been self-contained since 1932. Milking is by hand. The herd has been free from tuberculosis since 1932 while the history of the herd and three blood agglutination tests indicate its freedom from abortus infection. The first of these tests was carried out prior to July, 1934, and the second and third in November, 1937, and August, 1939.

At the time the experiments on calf-rearing were made, it was ascertained that the herd had been relatively free from udder troubles, and at a cultural examination of individual quarter milk samples in October, 1936, only three animals out of 18 were found to be infected and these had the disease in subclinical form.

The present series of observations cover practically a two years'

period, September, 1937, to August, 1939, and during this time 18 tests have been made of all cows in milk. Eleven of these tests were made at intervals as short as 7 to 16 days, in other cases the interval varied from 42 to 106 days.

For each test a sample of fore milk—usually about 50 c.c.—was taken at the afternoon milking from each quarter after rejecting the first stream. The milk was brought back to the laboratory, stored on ice or in the cooler weather at laboratory temperature and examined early next day. Ordinarily, the centrifuge deposit from 10 c.c. milk was plated in ox blood agar, half the deposit being put in one plate and one-twentieth of the deposit in a second plate. Gravity cream was similarly dealt with so that the first plate was sown with 1/20 c.c. and the second plate with 1/200 c.c. On three occasions the samples were allowed to stand at cool room temperature for a full 24 hours before they were used for cream and deposit plating. On the four last occasions the herd was tested, the examination was extended to include enrichment methods. For this purpose, (*a*) the deposit from 10 c.c. milk was transferred to 10 c.c. broth containing 1 per cent. horse serum and 1 per cent. glucose, incubated overnight, and if streptococci were seen microscopically, the culture was plated on the surface of ox blood agar; (*b*) 10 c.c. milk, to which a drop of 0·5 per cent. watery solution of brom-cresol purple had been added, was incubated at 37° C. overnight, when all milks showing definite acid production were examined microscopically and if streptococci were seen the culture was plated on ox blood agar. Streptococcus colonies were picked to serum broth and their reactions checked in 1 per cent. glucose broth, sucrose, salicin, mannite, inulin, raffinose, sodium hippurate, litmus milk and milk containing methylene blue at 1 : 20,000. Surface colonies on ox blood agar, which were greyish in colour, rather flattened and showing a trace of hæmolysis were usually identified as those of *Str. agalactiæ*. Deep colonies in ox blood agar plates showed narrow β-hæmolytic zones. Streptococci isolated from 22 quarters of 14 cows in the course of this enquiry were examined by the precipitin and agglutination tests by Dr. A. W. Stableforth and were found to be *Str. agalactiæ*, type Ib.

The observations have thrown some light on the relative value of the methods used for detecting *Str. agalactiæ*. In 29 instances where a milk sample contained this organism and where direct comparison could be made, positive results were secured as follows: incubation overnight followed by plating 24 times, plating from serum glucose broth 14 times, culture from cream 15 times, culture from deposit 11 times. Preliminary incubation of samples at 37° C. is therefore advantageous if it is desired to locate very slightly infected cows.

The final results obtained in the case of each animal are summarised in the chart, but the actual quarters infected are not shown. It is seen that out of 29 animals present in the herd during the

enquiry no less than 22 were found to be infected at one time or another, altogether in 49 quarters. Of these 22 animals, only two (Nos. 6 and 19) were clinically affected. In many cases infection was extremely light (*e.g.*, Nos. 3, 8, 9, 15, 17, 27 and 28) and might well have been missed if the cultural examination had not been intensive. Infection was revealed more frequently in the latter part of the lactation period. Infections when slight were not always detected in milk samples from the same quarter of the same cow at the different examinations. While, of course, this might have arisen through errors in sampling, the samples were always taken by one careful attendant and it is much more probable that light infections in various quarters of the udder were revealed only at certain tests ("cultural latency"). In about three-fifths of the tests of individual animals only one quarter was found to be infected: where infection was found at the same test in more than one quarter, the fact is indicated on the chart. These light infections caused no increase in centrifugable sediment or other change in the milk. The herd was visited three times yearly and the opportunity then taken to palpate the udders after milking; any slight abnormalities found in the texture or size of quarters were not obviously dependon light infections with streptococci.

Discussion.

There are at least two reasons why it has not been judged superfluous to give an account of events in this herd. Firstly, as stated earlier, it was desired to know if under the exceptionally favourable conditions it would be possible to repeat the observation of Minett, Stableforth and Edwards (1933) as to the formation of a group of cows free from *Str. agalactiæ*. Secondly, in view of the greater interest now being taken in mastitis, it was desired to make more widely known the views of Steck and others as to the practicability of controlling the chronic streptococcus form of the disease.

Repeated examination of individual cows has shown that herd BB is much more extensively infected than was at first believed, though it is of the "relatively free" status, as envisaged by Steck. Observations for a much longer period would have been required to determine whether the herd would eventually become completely free. Except in the early stages, however, practically no attempt was made to control the disease, apart from the elimination of two or three cows and the milking last of any showing more severe or more permanent infection.

It may be useful to consider in the light of knowledge acquired in recent years how infection is maintained in infected herds. The consensus of opinion at present is that the disease is spread from cow to cow solely by infective material coming in contact with the teats. Bendixen (1934) has amply illustrated the importance in this connection of injuries to the teat orifice. The evidence in favour

of the modern view that chronic streptococcus mastitis is contagious may be set down as follows: (1) the observation that the disease spreads to healthy cows when infected ones are placed in their midst, (2) the observation that the extent of the disease can be limited by measures designed to prevent milk-borne infection, (3) the low incidence of the disease among heifers compared with that among older cows, (4) the fact, ascertained by Stableforth (1938), that in most self-contained herds the infecting type of *Str. agalactiæ* is exactly the same serologically in all or most of the cows. In addition, there is the suggestion that the incidence of the disease in a herd may be related to the serological type of streptococci present in it. There can be little doubt that the evidence, especially on the first three points, is in need of further investigation. In connection with the third point it now seems that the incidence among heifers in their first lactation may be a good deal higher than was formerly believed, higher in fact in some herds than would be likely or possible if infection took place solely by the teat canal.

Reference may be made to the literature on this subject. Seelemann (1932) examined two series of cows, numbering 2,000 and 3,834 respectively, of various ages, all of which were certainly or probably infected with chronic streptococcus mastitis. The actual numbers of animals in each lactation period are not given, but it is stated that 3·5 and 13·7 per cent. respectively of those in the first lactation period were certainly infected. In the Annual Report for 1935 of the U.S. Bureau of Animal Industry it is stated that colostrum from each quarter of 36 first-calf heifers from a herd affected with mastitis was examined bacteriologically. No streptococci were found in the colostrum of 21 of these, while in the remaining 15 types resembling *Str. fæcalis* and streptococci other than *Str. agalactiæ* were found. Two of the animals, however, had become infected with *Str. agalactiæ* by the time a re-examination was made two and four weeks later. Stableforth, Edwards and Minett (1935) found that out of 125 heifers in four infected herds examined during their first lactation 23 (18·4 per cent.) were excreting *Str. agalactiæ*. McLean and Hewitt (1935) stated that 25 per cent. of the first-calf heifers examined by them in Victoria had streptococci in their milk within one month of calving. No information, however, is given of the total number of heifers examined or of the nature of the streptococci isolated.

Hucker and Harrison (1937)* reported on the incidence of infection in first-calf heifers belonging to three herds. Out of 68 heifers whose milk was tested during the first week of lactation 36 were said to be excreting

* There is nothing to show from the paper of Hucker and Harrison (1937) what proportion of the " mastitis streptococci " were *Str. agalactiae* and the authors' claims have to be considered in the light of this important reservation. The findings of Hucker (1937) also apply not to *Str. agalactiae* alone but also to the two other biochemical groups of mastitis streptococci. Actually from tables I and II of his report it appears that there were 21 cows and 24 heifers and calves ; from an examination of his data it can perhaps be assumed that *Str. agalactiae* was cultivated from nine and possibly from one more of the cows and from two and just possibly from one more of the younger animals. The quoted work of Hucker, of Hucker and Harrison and of McLean and Hewitt is a good illustration of the need not merely of a wider recognition of the differences among " mastitis streptococci," but also of the implications of these differences in practice, a need which it is the aim of the project of Little and Minett (1939) to satisfy.

" mastitis streptococci." Heifers from known infected cows were found to excrete streptococci rather more frequently than heifers from non-infected cows. Heifers originating from highly infected herds excreted streptococci much more frequently than ones from herds with a relatively low incidence of disease.

Some work has been done on the isolation of mastitis streptococci from the apparently healthy udder tissue of cows and younger animals after slaughter. Von Nida (1934) selected cows (age not stated) whose udders were clinically sound and which were giving normal milk as judged by naked-eye and microscopical examination. A piece of tissue was removed from the centre of the quarter and examined culturally. In this way udder tissue from 109 cows was examined and cultures of *Str. agalactiæ* were obtained from 21 of the udders. From a few other quarters streptococci of the " lacticus " type were isolated. Efforts of a similar nature were made by Hucker (1937)* (see page 272 footnote.) According to his report the udders of 24 cows which had passed through one or more lactation periods and of 21 virgin heifers and calves were examined. Although the organs were quite healthy, as judged by clinical and *post-mortem* inspection, " mastitis streptococci " were isolated from all the udders of the cows and from eight of the 21 udders of the younger animals.

There arises from the work quoted the important suggestion that infection with *Str. agalactiæ* may sometimes be acquired during calfhood and endure in a latent way to be revealed when lactation begins. In this connection, examination of the colostrum or of mammary tissue from heifers before or at first calving would be of particular interest, but quite obviously the whole matter urgently calls for enquiry, and until that has been carried out further discussion is pointless. In the meantime, it must be doubted whether the view is generally correct, that by a series of cultural examinations applied to the milk of individual cows it is possible to eradicate the disease relatively easily or quickly. This statement must by no means be held to imply that control measures based on such examinations should be discarded. Doubtless, some herds can be rendered free in this way, but the chief object in practice will be secured by the formation of a herd in which the number of clinically evident infections remains insignificant. Without trying to go into details, there would be required for this: (1) A periodic cultural examination of the milk of each cow so that latent cases easily demonstrable in this way and tending to persist may be segregated and perhaps treated. Suggestions as to procedure in diagnosis and control are given by Plastridge, Anderson, White and Rettger (1934), by S. J. Edwards (1934, 1938) and by Steck (1939). (2) Avoidance so far as possible of environmental influences which may lower the animal's resistance or in any other way interfere with the normal activity of the mammary tissue. The more precise definition of these influences will demand continuous careful observation and experiment in herds which are under the full control of the investigator, though some of these influences have, of course, been recognised for some time, *e.g.*, incomplete milking,

injuries to the teats. (3) Possibly, measures designed to control calfhood infection.

Summary.

Observations were made over a period of two years in a small herd lightly infected with streptococcus mastitis.

The control of the disease is discussed in a general way.

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DURATION OF IMMUNITY INDUCED IN CALVES BY BCG

II.—Resistance Induced by Multiple Doses of the Vaccine.

By J. B. Buxton, R. E. Glover,
T. Dalling and T. J. Bosworth.
Institute of Animal Pathology, University of Cambridge.

The present article represents the final stage in the investigation into the efficacy of BCG vaccine for cattle maintained under laboratory conditions. Following the preliminary report on the relative merits of various routes of administering BCG vaccine in evoking a resistance to a severe method of test, *viz.*, the intravenous inoculation of virulent bovine tubercle bacilli (Buxton and Griffith, 1931), it was shown that calves receiving two doses of 10 mg. and 100 mg. respectively of BCG given intravenously with an interval of three weeks between the injections, were solidly immunised against an infection induced by the feeding of virulent cultures of tubercle bacilli six months after the protective inoculation. The immunity commenced to wane after the lapse of twelve months. Further experiments indicated that re-vaccination with BCG by the same route six, nine or twelve months respectively after the preliminary stimulus restored the immunity, but the protection was complete in a smaller proportion of calves than in the preceeding series (Griffith, Buxton and Glover, 1931 ; Buxton, Glover and Griffith, 1934).

The results of these experiments were disappointing to the extent that a complete protection of every calf was not secured : on the other hand, the lesions in the vaccinated animals when compared with the controls, were appreciably restricted in their extent. It is true that in some instances the test organisms succeeded in establishing themselves in a few glands of the alimentary tract, but apparently the process was not active and the impression was gained that the lesions were undergoing retrogression. Thus, in the control calves caseation was the most common change which was noted, whereas in the vaccinated animals many of the lesions seemed to be undergoing fibrosis. Taken as a whole it was considered that the evidence which had been obtained was sufficient to justify a further series of experiments on the duration of the immunity induced in calves by repeated doses of BCG given at six-monthly intervals. It seemed to be of some importance to ascertain whether any material breakdown in immunity similar to that reported by Watson and his colleagues (1930) would occur in older animals exposed to a fairly continuous infection over a longer period than in the preceding series.

It has been suggested that in this country BCG or a similar vaccine might be of value in those herds which do not lend themselves to the eradication of tuberculosis by systematic testing and the elimination of reactors. The application of the vaccine, however, is not entirely simple since the circumstances vary materially as between

individual herds. On some farms it is possible to separate the calves at, or soon after, birth, and to segregate them in such a way that they will stand a reasonable chance of remaining free from infection during the first 18 months of their lives. At the end of this period, they are probably drafted into the main herd, where they may immediately run the risk of exposure to a heavy and continued infection. In other cases no facilities exist for the proper isolation of the calves, and they are, therefore, liable to come into contact with infected adult stock and so require protection at a very early date.

The experiments which are to be described can be conveniently grouped into three sections, each of which will be considered separately. They comprise (*a*) cattle vaccinated with BCG every six months for a period of three years, kept under conditions of rigid isolation from exposure to tuberculous infection, and finally infected with a single dose of virulent tubercle bacilli given by the mouth; (*b*) cattle vaccinated with BCG every six months and exposed to multiple infections with virulent bovine tubercle bacilli by the mouth shortly after the first dose of vaccine, the infective dose being repeated at intervals: and (*c*) cattle vaccinated with BCG every six months, isolated for a period of 18 months and then exposed to infection as group (*b*).

Experiment (*a*) is a logical extension of the previous series and was designed to test the duration of immunity over a fairly long period under purely laboratory conditions. Experiments (*b*) and (*c*), on the other hand, were devised with the object of reproducing as far as possible the conditions which might be encountered in naturally infected herds of cattle. In this connection, it is proper to point out that in these experiments the conditions laid down by Calmette and Guérin, *viz.*, that the successful suppression of tuberculosis by BCG is dependent to some extent upon the simultaneous application of suitable hygienic measures, were not completely observed.

Animals Used in the Experiments.

The majority of the calves were of the Ayrshire breed and were purchased from tubercle-free herds in South-West Scotland. On arrival at the Institute they were immediately isolated and were subjected to three preliminary tuberculin tests at intervals of approximately six weeks. All the animals were negative to these tests. The majority was then allotted to the vaccinated groups but a few were reserved to act as controls and were supplemented by a number of tuberculin-negative cattle purchased locally or bred at the Institute. The total number of calves available for the three experiments was 57, of which 40 were vaccinated with BCG and 17 were reserved as controls. The allocation of the calves to the three different experiments is dealt with in the appropriate sections.

Before the details of each experiment are given, it may not be out of place to refer here to the conditions under which the animals were maintained. Prior to the administration of the test dose the

calves were isolated on a remote part of the Institute where they were not likely to be exposed to chance infection. During this period they received one or more doses of BCG vaccine according to the group in which they had been placed. Thus the calves in experiments (*a*) and (*c*) were allowed to remain in isolation for 36 and 18 months respectively, whereas those in experiment (*b*) were immediately housed in the two buildings which had harboured the infected animals in the previous series of experiments. One of these buildings contained 14 boxes, each isolated from its neighbour but all opening on to a common corridor, while the other was a closed shed divided into six temporary compartments. The animals were distributed between the two buildings; those in the first-mentioned were in semi-isolation, whereas those in the second were in relatively close contact. As far as possible one vaccinated and one control animal inhabited each box in the first building, but frequent changes were made, the animals being transferred from one division to another and from one building to another. About twelve months after the experiment had commenced it was found that the animals were becoming unduly crowded. It was, therefore, decided to construct an open yard adjacent to and in communication with the second building. Under these conditions, the cattle had direct access to the yard, where controls and vaccinated animals were allowed to intermingle freely. At the end of 18 months the cattle in experiment (*c*) were brought into the two infected buildings and they in turn had every opportunity of mixing, not only with their own controls, but also with those of experiment (*b*).

The animals under experiment (*a*), on the other hand, were isolated from any chance of tuberculous infection during the whole period of vaccination (three years). They were then brought into the isolation boxes to receive their infective dose and remained there until the termination of the experiment.

Duration of Immunity.

(*a*) Exposure to Infection Three Years after First Dose of Vaccine.

For the first experiment twelve calves were separated from the rest of the herd. The preliminary dose of BCG comprising 100 mg. was given intravenously to ten of the animals between April 2nd and April 24th, 1935, while two were kept unvaccinated to act as controls. With the exception of two calves in the vaccinated group which died before the termination of the experiment, the protected calves received reinforcing doses of vaccine at approximately six-monthly intervals, five being injected with 50 mg. and three with 100 mg. The total number of doses given to each animal was six. The method of preparation of the vaccine has been fully described in a previous article (Buxton and Griffith, 1931).

Two animals (212 and 218) died at an early age.

No. 212 received 100 mg. of BCG on April 17th, 1935 and 50 mg. on October 14th, 1935. Five days later the animal was very ill and showed

obvious signs of respiratory distress. It died during the course of the day. The *post-mortem* examination showed a marked engorgement of the lymphatic glands of the head and thoracic cavity. The lungs were enormously distended and the interlobular spaces were filled with a gelatinous exudate.

No. 218 received one dose of BCG vaccine on April 15th, 1935. It died from traumatic pericarditis on July 21st, 1935.

On April 24th, 1938, the two control animals were tested with tuberculin with negative results. Three days later, *i.e.*, on April 27th, 1938, and approximately three years after the commencement of the experiment, the eight vaccinated animals which remained, together with the two controls, were transferred to the infected building where they were housed in individual compartments. Each received an infecting dose consisting of 5 mg. of a mixture of six strains of virulent bovine tubercle bacilli grown separately on plain egg agar for ten days, weighed and suspended in normal saline solution. The dose selected, in about 3 c.c. of normal saline solution, was pipetted into the mouth of each calf. The strains were tested on rabbits and guinea-pigs prior to the commencement of the experiment and were found to be of the standard virulence of the bovine type.

The animals were killed between November 11th and December 16th, 1938.

Controls.

No. 269 was killed on November 29th, 1938, *i.e.*, 216 days after the test dose.

The right submaxillary gland was enlarged to the size of a duck egg and filled with thick, yellowish caseo-pus. The right mediastinal gland showed a caseo-calcareous lesion the size of a bean. The liver contained 20 encapsulated lesions up to the size of a hazel nut, while the portal gland was enlarged and beset with pin-point lesions. The mesenteric, cæcal and colic glands were grossly affected. The mesenteric chain showed an uncountable number of caseo-purulent lesions of the size of golf balls, while many were the size of a large pea or bean. Guinea-pigs inoculated with various glands died of generalised tuberculosis.

No. 300 was killed on November 28th, 1938, *i.e.*, 215 days after the test dose.

Both pharyngeal glands were enlarged to four times the normal and almost entirely replaced with tuberculous tissue containing caseo-calcareous centres. The left submaxillary gland was also enlarged and about one-half the substance was tuberculous. In the long mediastinal gland there was a caseo-calcareous lesion about the size of a bean. The mesenteric glands were very extensively affected. Several foci up to the size of a walnut were seen, while innumerable smaller lesions from the size of a lentil to a bean were scattered throughout the chain. Five colic glands were affected. Guinea-pigs inoculated with suspensions from various glands died of generalised tuberculosis.

The eight vaccinated calves were killed 216, 218, 220, 222, 229 (2), 232 and 233 days respectively after the resistance test dose. Five (see Table I) were entirely free from visible lesions and the absence

of viable tubercle bacilli was confirmed by guinea-pig inoculation. In the sixth (221) there were no macroscopic lesions of tuberculosis, but each of two guinea-pigs injected with a mixed suspension of head and thoracic glands died of generalised tuberculosis.

In the remaining two animals (216 and 220) the lesions were not extensive.

No. 216. Lesions were found which were not typically tuberculous in appearance. Both pharyngeal glands were slightly enlarged. The right gland contained a pea-sized lesion composed of a thick fibrous tissue wall enclosing a viscous yellowish pus. The left gland contained a similar but smaller lesion. Three mesenteric glands also contained lesions varying from a pea to an oat grain containing a thick pus. Although no acid-fast bacilli were seen in these lesions, guinea-pigs inoculated with pus from the head and mesenteric glands respectively, died of generalised tuberculosis.

No. 220. Showed macroscopic lesions in the right pharyngeal, one mesenteric and two colic glands. The foci were not larger than a pinhead and were calcareous. A few acid-fast bacilli were seen in smears. Two guinea-pigs injected from the affected pharyngeal gland and two from the abdominal glands died of generalised tuberculosis.

The results of these experiments indicate a marked degree of resistance on the part of the vaccinated calves as compared with the controls. In five out of the eight there were no macroscopic lesions and guinea-pig tests were negative. In one, the organs and glands were free from visible tuberculosis but guinea-pigs died or were killed with generalised tuberculosis. In the remaining two animals the lesions were very slight as compared with the controls, in which extensive changes were found not only in the glands associated with the alimentary tract but also in the thoracic and, in one case, the abdominal cavity.

(*b*) Exposure to Infection Immediately after First Dose of Vaccine.

For this experiment 30 calves (20 vaccinated and 10 controls) were used. The first dose of BCG, comprising 100 mg., was given intravenously between April 15th and 17th, 1935. With the exception of two calves which died at an early stage of the experiment, all the vaccinated animals received a reinforcing dose of BCG (100 mg. in the case of ten calves, and 50 mg. in the case of eight). The reinforcing doses were given at intervals of approximately six months until each animal had received six doses.

On June 21st, 1935, the vaccinated animals, together with ten tuberculin negative controls, were brought into the infected houses where they were grouped in pairs, the vaccinated calves being distributed as evenly as possible among the controls. All these animals then received an infective dose prepared from a mixture of the six strains of virulent bovine tubercle bacilli used in experiment

TABLE I.

B.C.G. Dates.	B.C.G. Mg.	Test Dose virulent TB. Date.	Test Dose virulent TB. Mg.	Duration of Life after Test Dose Days.	Post-mortem Findings. Head Glands.	Thoracic Glands.	Abdominal Glands.	Result.	Guinea-pig Tests.	Result.	No. of Calf.
		27.4.38	5·0	K232	—	—	—	—	H. —— T. O A. ——	—	215
April, 1935	100	27.4.38	5·0	K229	? +	—	? +	?+	H. + T. O A. +——	++	216
14.10.35	50										
5.5.36	50	27.4.38	5·0	K233	+	—	+	+	H. ++ T. O A. ++	++	220
12.11.36	50										
25.5.37	50	27.4.38	5·0	K229	—	—	—	—	H. —— T. O A. ——	—	223
5.11.37	50										
		27.4.38	5·0	K222	—	—	—	—	H. T. A. ——	—	224
April, 1935	100	27.4.38	5·0	K216	—	—	—	—	H. —— T. A. ++	++	221
October, 1935	100										
5.5.36	100	27.4.38	5·0	K218	—	—	—	—	H. —— T. —— A. ——	—	222
12.11.36	100										
25.5.37	100	27.4.38	5·0	K220	—	—	—	—	H. —— T. O A. ——	—	232
5.11.37	100										
None		27.4.38	5·0	K216	+	++	++++	++++	H. T. A. ++	+++	269 (Control)
None		27.4.38	5·0	K215	++	+	++++	++++	H. ++ T. ++ A. ++	+++	300 (Control)

[illegible] ... inguinal and/or mesocolic + = Each sign represents one guinea-pig.

(*a*). Eight vaccinated and five controls each received 3 mg., while the remainder, ten vaccinated and five controls, were given a smaller dose of 1 mg. The latter group received two additional doses, each of 1 mg., at intervals of approximately four weeks. Similar infective doses were repeated at approximately six-monthly intervals (see Tables II, III and IV). On the next two occasions a single dose of 3 mg. was given to all animals and finally two doses of 5 mg. The spacing of the infective doses in relation to the reinforcing injections of BCG vaccine was altered from time to time, being given either immediately after the injection of the vaccine, or midway between the two doses, or shortly before a reinforcing dose.

As already explained, when about 18 months had elapsed the major proportion of the animals, comprising both vaccinated and controls, was collected into one group with those of experiment (*c*) so that the animals were allowed to come into free contact. The chances of infection in the vaccinated group were, therefore, fourfold, *viz*. (1) by the direct introduction of virulent tubercle bacilli by the mouth ; (2) by contact infection in pairs ; (3) by contact infection as a group ; and (4) by indirect contact with infected standings, manure, etc. It can thus be seen that the vaccinated animals were subjected to a very severe test.

Two animals died at an early stage of the experiment.

Calf 237, vaccinated with 100 mg. BCG on April 4th, 1935, received an infective dose of 3 mg. by the mouth on June 21st, 1935. A second dose of 100 mg. of vaccine was given on October 7th, 1935. Twenty-four hours later the animal was found dead.

The *post-mortem* examination showed a very marked engorgement of the lungs. On section they showed distention of the interstitial tissue with exudation of an œdematous fluid. The glands throughout the body, but in particular the mediastinal, bronchial, pharyngeal and submaxillary, were enlarged, spongy and hæmorrhagic. No lesions of tuberculosis were found in the organs or glands.

Three guinea-pigs inoculated with a mixture of different glands were killed 115 days later and were free from tuberculosis.

Calf 241 received 100 mg. BCG on April 8th, 1935, and a test dose of virulent tubercle bacilli on June 21st, 1935. On July 25th, 1935, the animal was depressed and refused to eat. During succeeding days it lost condition rapidly and showed a well-marked respiratory disturbance. It was found dead on July 31st, 1935.

At the *post-mortem* examination lesions were found which were confined to the thoracic cavity. The lungs were affected with pneumonia, the anterior lobes on each side being very firm and beset with yellow lesions about the size of a bean. On section each contained a viscous white pus. The middle and posterior lobes showed areas of acute emphysema alternating with areas of broncho-pneumonia. The associated lymph glands were enlarged but otherwise not visibly altered. A smear from a lung nodule showed numerous Gram-positive diplococci. No lesions of tuberculosis were found in any of the organs or glands. The pulmonary lesions were found to be due to a traumatic reticulitis.

Guinea-pigs were inoculated with suspensions from various tissues including mesenteric, pharyngeal, submaxillary and bronchial glands, and portions of consolidated lung. When killed on November 15th, 1935 (107 days) all the animals were free from tuberculosis.

Control Group.

The animals in this group were killed from 156 to 160 weeks after the commencement of the experiment. There were marked variations in the nature of the lesions in the control group ranging from one animal which was entirely free from macroscopic evidence of the disease to those which showed fairly widespread lesions. The extent of the changes found at *post-mortem* examination and the results of the inoculation of guinea-pigs with various glands and organs which are set out briefly in Tables II, III and IV can be conveniently divided into four groups, *viz.* (*a*) animals showing no lesions ; (*b*) animals with slight lesions in the glands of the head or abdominal cavity only ; (*c*) animals with lesions in all the glands associated with the alimentary tract ; and (*d*) animals with lesions in both the abdominal and thoracic cavities.

(*a*) One animal (317) was entirely free from visible lesions. Guinea-pigs were injected with suspensions of the pharyngeal and mesenteric glands. The former died 14 days later and showed no evidence of tuberculosis ; the latter were killed at the 36th day and showed advanced tuberculosis.

(*b*) Two animals (273 and 276) showed very slight lesions only. In 273 both pharyngeal glands were enlarged to the size of hens' eggs and contained caseous lesions. Both tonsils were much enlarged with caseous material in the crypts. There were no lesions in any other glands or organs. In 276 only one gland in the cæco-colic group was obviously affected, being slightly enlarged and containing two pinhead-sized lesions. Guinea-pigs inoculated with suspensions from the head and abdominal glands of 273 died of generalized tuberculosis, while in 276 the abdominal glands alone proved infective for guinea-pigs.

(*c*) Five animals (264, 274, 297, 307 and 327) showed some widespread lesions of the alimentary tract. They varied in extent from a pea-sized caseo-calcareous lesion in a pharyngeal gland and a similar focus in one mesenteric gland (307), up to much more extensive changes (297) in which both pharyngeal glands were enlarged to the size of duck eggs and almost completely replaced with caseo-calcareous material, while four mesenteric glands showed bean-sized caseo-calcareous lesions. Guinea-pigs in this instance inoculated with various tissues, died or were killed with generalised tuberculosis.

(*d*) Two animals (312 and 314), in addition to lesions in glands associated with the alimentary tract, also showed tuberculous changes in the thoracic cavity. In one case both the bronchial and mediastinal glands were affected and in the other a mediastinal gland only.

The lesions in this group of control animals were less extensive than had been anticipated and were certainly not so widespread as

TABLE II.—CONTROLS.

Test Doses. Dates.	Virulent TB. Mg.	Duration of Life after 1st Test Dose. Weeks.	Post-mortem Findings. Head Glands.	Thoracic Glands.	Abdominal Glands.	Result.	Guinea-pig Tests.	Result.	No. of Calf.
21.6.35	3·0	156	+	—	+	+	H. ++* T. O A. ++	++	264
12.11.35	3·0	160	++	—	+	+	H. ++ T. O A. ++	++	274
8.6.36	3·0								
25.2.37	3·0	156	++++	—	+	++	H. ++ T. O A. ——	+	297
18.8.37	3·0								
27.10.37	5·0	159	++	+	—	+	H. ++ T. ++ A. ++	+++	314
17.2.38	5·0								
		160	++	—	++	++	H. ++ T. O A. ++	++	327
		159	++	—	—	+	H. ++ T. O A. ++	++	273
21.6.35	1·0								
19.7.35	1·0								
20.8.35	1·0	158	—	—	+	+	H. —— T. —— A. ++++	+	276
12.11.35	1·0								
9.12.35	1·0								
7.1.36	1·0								
8.6.36	1·0	157	+	—	+	+	H. +— T. O A. ++	++	307
6.7.36	1·0								
6.8.36	1·0								
25.2.37	3·0								
18.8.37	3·0	160	—	++	++	+++	H. —— T. ++ A. ++	++	312
27.10.37	5·0								
17.2.38	5·0								
		159	—	—	—	—	H. —— T. O A. ++	+	317

* Each sign represents one guinea-pig.

those which were obtained in the control calves in the previous series of experiments.

Vaccinated Animals.

The animals in this group were killed at about the same period as the controls. The vaccinated calves can be divided into two groups, *viz.* (1) those which received a single infective dose between each injection of vaccine, and (2) those which in the early stage of the experiment received a series of smaller doses at more frequent intervals.

Group 1 comprised eight animals of which five (213, 217, 236, 238 and 242) were vaccinated at six-monthly intervals with 100 mg. of BCG, while three (248, 250 and 253) received an initial dose of 100 mg. and 50 mg. on subsequent occasions. The total number of injections in each group was six, spread over a period of three years. The test doses of virulent tubercle bacilli were given to the vaccinated animals concurrently with the controls. The results are set out in Tables III and IV and are grouped in the same manner as in the controls.

(*a*) Two animals (213 and 253) were apparently free from tuberculosis. In 213 no lesions of any description were found and guinea-pigs inoculated with various glands remained unaffected. In 253 a pharyngeal gland was replaced by firm tissue containing pinhead gritty centres. No acid-fast bacilli were found in smears, however, and guinea-pigs inoculated with this and other glands remained free from tuberculosis.

(*b*) In three animals (217, 238 and 250) lesions were found in the mesenteric glands only. They were of moderate extent, the maximum number affected being seven, but they varied in size from a duck's egg to a millet seed. In each instance, guinea-pig inoculations showed that the mesenteric glands were affected. None of the other glands was tuberculous except in the case of 217 in which a pharyngeal gland induced generalised tuberculosis in guinea-pigs.

(*c*) Three animals (236, 242 and 248) showed some extensive lesions. In two instances (236 and 248) the mesenteric glands were widely affected, while in all three the glands of the head were also tuberculous.

(*d*) There was no animal in this series which showed tuberculosis of the thoracic glands.

Group 2 was composed of ten animals of which five (214, 219, 226, 228 and 240) were injected with 100 mg. doses of BCG, while five (229, 245, 246, 249 and 252) received an initial injection of 100 mg. followed by 50 mg. doses. The results of these experiments are also summarised in Tables III and IV.

(*a*) Three animals (214, 219 and 245) were free from visible lesions of tuberculosis. In one instance (214) the absence of viable tubercle bacilli was confirmed by guinea-pig inoculations, but in 219 suspensions from the thoracic and abdominal glands induced

TABLE III.—VACCINATED.

B.C.G.		Test Dose virulent TB.		Duration of Life after Test Dose.	Post-mortem Findings.			Result.	Guinea-pig Tests.	Result.	No. of Calf.
Dates.	Mg.	Dates.	Mg.	Weeks.	Head Glands.	Thoracic Glands.	Abdominal Glands.				
		21.6.35	3·0	153	++	—	+++	+++	H. ++ T. O A. ++	++	248
		12.11.35	3·0								
		8.6.36	3·0								
		25.2.37	3·0	159	—	—	+	+	H. — — T. O A. ++	+	250
*April, 1935	100	18.8.37	3·0								
		27.10.37	5·0								
†October, 1935	50	17.2.38	5·0	153	? +	—	—	? +	H. — — T. — — A.	—	253
‡May, 1936	50										
9.11.36	50			159	++	++	++	+++	H. + — T. ++ A. + —	+	229
25.5.37	50										
		21.6.35	1·0								
5.11.37	50	19.7.35	1·0	153	—	—	—	—	H. ++ T. ++ A. ++	+++	245
		20.8.35	1·0								
		12.11.35	1·0								
		19.12.35	1·0								
		7.1.36	1·0								
		8.6.36	1·0	154	—	—	+	+	H. T. — — A. ++	+	246
		6.7.36	1·0								
		6.8.36	1·0								
		25.2.37	3·0	159	++	—	+	++	H. ++ T. O A. ++	++	249
		18.8.37	3·0								
		27.10.37	5·0								
		27.2.38	5·0								
				160	—	—	+	+	H. T. ++ A. ++	++	252

Dates: * 11.4.35 245; 15.4.35 229, 246, 248, 249, 250, 252, 253. † 14.10.35 229, 245, 246, 248, 249, 250; 16.10.35 252, 253.
‡ 5.5.36 245, 246, 248, 252, 253; 12.5.36 229, 249, 250.

TABLE IV.—VACCINATED.

B.C.G. Dates.	B.C.G. Mg.	Test Dose virulent TB. Dates.	Test Dose virulent TB. Mg.	Duration of Life after 1st Test Dose. Weeks.	Post-mortem Findings. Head Glands.	Thoracic Glands.	Abdominal Glands.	Result.	Guinea-Pig Tests.	Result.	No. of Calf.
				160	—	—	—	—	H. T. A. — —	—	213
		21.6.35	3·0	160	—	—	+	+	H. ++ T. O A. ++	++	217
		12.11.35	3·0								
		8.6.36	3·0	157	+	—	++	++	H. ++ T. O A. ++	++	236
		25.2.37	3·0								
		18.8.37	3·0	153	—	—	++	+	H. O T. O A. ++	+	238
* April, 1935	100	27.10.37	5·0								
† October, 1935	100	17.2.38	5·0	124	+	—	+	+	H. + T. O A. +	++	242
‡ May, 1936	100										
9.11.36	100			156	—	—	?	? +	H. T. — — A. — —	—	214
25.5.37	100										
5.11.37	100	21.6.35	1·0	124	—	—	—	—	H. — T. + A. +	++	219
		19.7.35	1·0								
		20.8.35	1·0								
		12.11.35	1·0								
		9.12.35	1·0								
		7.1.36	1·0	159	+	—	+++	++	H. ++ T. O A. ++	++	226
		8.6.36	1·0								
		6.7.36	1·0								
		6.8.36	1·0								
		25.2.37	3·0	160	++	—	++++	+++	H. ++ T. O A. ++	++	228
		18.8.37	3·0								
		27.10.37	5·0								
		17.2.38	5·0								
				153	++	++	++	+++	H. ++ T. ++ A. ++	+++	240

* 8.4.35 236, 238, 240, 242; 15.4.35 226, 228; 17.4.35 214, 217; 24.4.35 213, 219.
† 7.10.35 236, 238, 240, 242; 14.10.35 213, 214, 226, 228; 16.10.35 217, 219.
‡ 5.5.36 214, 217, 219, 226, 228; 12.5.36 213, 236, 238, 240, 242.

generalised tuberculosis in guinea-pigs, and in 245 all the inoculated guinea-pigs showed generalised tuberculosis.

(*b*) In two animals (246 and 252) the lesions were slight and were confined to two and four mesenteric glands respectively. In 246 only the guinea-pig inoculated with the mesenteric gland suspensions proved tuberculous, whereas in 252 a mixed suspension of thoracic and head glands was also infective.

(*c*) In three animals (226, 228 and 249) the lesions were more extensive and were located in the head glands as well as those of the alimentary tract. In two out of three both pharyngeal glands were severely affected, while the number of mesenteric glands showing lesions varied from three (249) up to 17 (228).

(*d*) In two animals (229 and 240) the lesions were widespread and moderately severe. Foci were found not only in glands in the head and mesenteric groups, but also in the thoracic cavity. In 229 the right lung was slightly affected and the mediastinal glands showed caseous centres, while in 240 there were a half-dozen pulmonary lesions and the associated glands were fairly extensively affected.

The lesions in the control group of calves were much less extensive than had been anticipated. The experience obtained in the previous series of experiments (Griffiths, Buxton and Glover, 1931) in which generalisation had commenced in the controls six months after the test dose had seemed to indicate that the tests used and the route of infection were sufficiently reliable to induce a progressive form of the disease. It was thought that the conditions of close cohabitation, coupled with the administration of repeated doses of virulent tubercle bacilli in the present series, should lead to the formation of widespread lesions, but such did not prove to be the case.

The explanation of the failure to obtain the desired results is not very apparent. It may be mentioned, however, that the controls available for this experiment were rather more mature than those selected on the previous occasion. It is suspected that susceptibility to infection induced by the alimentary tract diminishes with age and the comparatively trivial nature of the lesions in some of the controls may be explained on these grounds ; moreover, the establishment of virulent tubercle bacilli in a single gland or group of glands may prevent the subsequent entry of infective organisms so that the repeated feeding of cultures by the mouth may not have produced the summation of effects which it was hoped would be secured.

Taken as a whole, there is some indication that the vaccinated animals, when compared with the controls, showed a slight degree of protection, but it is doubtful whether it can be regarded as of any material significance.

(*c*) Exposure to Infection Eighteen Months after First Dose of Vaccine.

The number of calves available for this experiment was ten vaccinated and five controls. The first dose of vaccine, comprising 100 mg. of BCG, was given by the intravenous route in April, 1935,

and thereafter further injections of either 50 or 100 mg. were made at approximately six-monthly intervals until the animals had received three doses. During this period they had not been in contact with any tuberculous cattle. The controls were tested periodically with tuberculin and invariably gave negative results.

On July 1st, 1936, *i.e.*, approximately 80 weeks after the third dose of vaccine, the ten protected animals together with the five controls, received a test dose by the mouth ; four vaccinated calves and three controls received 3 mg., and the remainder 1 mg. They were then allowed to mix with the animals in group (*b*), with whom they cohabited until the termination of the experiment. From this period they were treated in a similar way to the preceding group, *i.e.*, reinforcing doses of vaccine were given at approximately six-monthly periods and test doses of virulent tubercle bacilli by the mouth in the intervening intervals (Tables V and VI).

The animals were slaughtered about three years after the commencement of the experiment and were post-mortemed with the following results :—

(1) *Control Group.*—The results of the examination of these animals are summarised in Table V. On the whole, the disease was much more severe than in the previous group, every animal showing fairly widespread lesions. In one animal (292) the glands of the head appeared to have escaped infection, but there was very widespread disease in the mesenteric glands while the mediastinal glands showed two pea-sized caseo-calcareous lesions. The animal most severely affected was 303, which presented widespread lesions in the glands of the head, thorax and abdomen, and also showed two caseo-purulent lesions in the lungs. In the remaining three calves (263, 286 and 313) the lesions ranged from 10 to 40 caseo-calcareous foci in the mesenteric glands and lesions of varying extent in the pharyngeal, bronchial and mediastinal glands.

(2) *Vaccinated Animals.*—With two exceptions, the protected animals showed less extensive lesions than the controls.

(*a*) Two animals (227 and 247) were entirely free from visible lesions, while two (233 and 243) each showed an atypical focus in a single mesenteric gland. Guinea-pigs inoculated from 227 remained free from tubercle bacilli, but in the other three a proportion of the guinea-pigs receiving glandular suspensions succumbed with generalised tuberculosis. With the exception of 243, in which the glands of the head were affected, viable tubercle bacilli were restricted to the mesenteric glands.

(*b*) Two animals (234 and 235) showed lesions confined to the mesenteric group of glands. The maximum number affected was seven, but the foci were relatively large, varying in size from an orange downwards.

(*c*) In two animals (230 and 231) the head glands were severely affected. In 230, however, only a single mesenteric gland was tuberculous, whereas in 231 the lesions in this situation were very severe.

TABLE V.—CONTROLS.

Test Doses. Dates.	*Virulent TB.* Mg.	*Duration of Life after 1st Test Dose.* Weeks.	*Post-mortem Findings.* *Head Glands.*	*Thoracic Glands.*	*Abdominal Glands.*	*Result.*	*Guinea-pig Tests.*	*Result.*	*No. of Calf.*
6.7.36	3·0	108	++	++	+++	++++	H. ++*	+++	263
25.2.37	3·0						T. +		
							A. +		
18.8.37	3·0	108	—	+	++++	+++	H. O	++	292
27.10.37	5·0						T. ++		
							A. ++		
17.2.38	5·0	110	++	++	+++	+++	H. ++	+++	313
							T. +		
							A. +		
1.7.36	1·0	107	+	+	++++	+++	H. ++	+++	286
6.8.36	1·0						T. ++		
2.9.36	1·0						A. ++		
25.2.37	3·0								
18.8.37	3·0	109	+++	+++	+++	++++	H. +	+++	303
27.10.37	5·0						T. +		
17.2.38	5·0						A. ++		

H = Pharyngeal and/or submaxillary.
T = Bronchial and/or mediastinal.
A = Mediastinal and/or caeco-colic.

O = Not done.
* Each sign represents one guinea-pig.

TABLE VI.—VACCINATED.

B.C.G.		Test Dose virulent TB.		Duration of Life after Test Dose. Weeks.	Post-mortem Findings.			Result.	Guinea pig Tests.	Result.	No. of Calf.
Dates.	Mg.	Dates.	Mg.		Head Glands.	Thoracic Glands.	Abdominal Glands.				
				109	++	—	+	+	H. ++ T. O A. ++	++	230
		1.7.36	3·0	110	+	—	+++	++	H. ++ T. O A. ++	++	231
		25.2.37	3·0								
		18.8.37	3·0	110	—	—	? +	±	H. O T. O A. ++	+	233
*April, 1935	100	27.10.37	5·0								
† October, 1935	100	17.2.38	5·0	109	—	—	++	+	H. O T. —— A. ++	+	234
5.5.36	100										
		1.7.36	1·0								
9.11.36	100	6.8.36	1·0	109	—	—	+	+	H. O T. O A. ++	+	235
		2.9.36	1·0								
25.5.37	100	25.2.37	3·0								
		18.8.37	3·0								
5.11.37	100	27.10.37	5·0	109	—	+++	+++	+++	H. O T. ++ A. ++	++	239
		17.2.38	5·0								
April, 1935	100	1.7.36	3·0						H. ——		
		25.2.37	3·0								
October, 1935	50	18.8.37	3·0	111	—	—	—	—	T. ——		227
		27.10.37	5·0								
5.5.36	50	17.2.38	5·0						A. ——		
9.11.36	50										
				110	—	—	? +	±	H. ++ T. —— A. ++		243
25.5.37	50	1.7.36	1·0								
		6.8.36	1·0								
5.11.37	50	2.9.36	1·0								
		25.2.37	3·0	110	++	+++	+++	++++	H. ++ T. + A. +	+++	244
		18.8.37	3·0								
		27.10.37	5·0								
		17.2.38	5·0								
				111	—	—	—	—	H. O T. O A. ++	+	247

* 2.4.35 230, 231, 233; 8.4.35 234, 235, 239; 10.4.35 227; 11.4.35 243, 244, 247. † 7.10.37 230, 231, 232, 234, 235, 239;

(*d*) In two animals (239 and 244) lesions were also found in the thoracic cavity and were very severe throughout the body. In 239 the head glands were not affected but there were about two dozen caseo-calcareous pulmonary lesions and the associated glands contained foci. There was a severe tuberculous peritonitis, the liver showed scattered tubercles and the mesenteric glands were widely affected. In 244 the lesions were similar in extent and severity but the head glands were also much enlarged and caseous.

Examination of the results suggests that the vaccine may have induced a slightly higher degree of resistance than in the previous group, since 40 per cent. showed a complete immunity as judged by the absence of macroscopic lesions (10 per cent. by guinea-pig test). In the remaining 60 per cent. the lesions were a little less extensive than in the controls, but the margin which separated the two groups was not very significant. There was no appreciable difference as between the 50 mg. and 100 mg. doses of BCG.

Tuberculin Tests.

In previous articles (Griffiths, Buxton and Glover, 1931 ; Buxton, Glover and Griffith, 1934) brief references have been made to the results of serial tuberculin tests in calves vaccinated with BCG. It was found that seven days after a primary intravenous inoculation, a marked sensitivity to tuberculin developed, reaching its maximum by about the third week and remaining at a high level for a period of from three to six months. Provided a reinforcing dose of vaccine was not given, a steady decline in the skin sensitivity to tuberculin was noted, and in many instances tuberculin tests applied at the end of twelve months gave negative results, although a few animals were still slightly allergic after 18 months. Re-vaccination of calves six, nine and twelve months after an initial inoculation restored the sensitivity of the skin to its original level, but the subsequent decline was more rapid since many animals were almost completely tuberculin negative six months after the reinforcing dose.

The present experiments have enabled us to study in greater detail the reactions of vaccinated calves to tuberculin on a relatively larger number of animals (36) over a period of three and a half years. The environmental conditions were not the same for all these animals since, although the cattle were invariably vaccinated at six-monthly intervals, one group was protected from tuberculous infection for three years, one group for 18 months, while the third group was exposed to infection shortly after the primary injection of vaccine.

It will be convenient, therefore, to consider the three groups separately, since contact with virulent bovine tubercle bacilli seemed to influence the tuberculin response and has, therefore, to be taken into account. The tuberculin tests were applied at irregular intervals but in most instances the animals were tested between successive injections of vaccine and between successive doses of virulent tubercle bacilli. It is not within the scope of this article to discuss each

animal in detail, but an attempt has been made to summarise the information obtained by considering the average increase in skin measurement, *i.e.*, the difference between the initial and final readings for the individual calves and then obtaining a mean for the group.

(1) *Animals Protected from Infection until the Termination of Vaccination.*—Five weeks after the initial inoculation of BCG all the animals had become highly allergic, the average rise in skin measurement being 32 mm. (Chart 1). By the fifteenth week the dermal response for the group had declined to 8 mm. The second dose of vaccine given at the twenty-sixth week induced a marked rise in skin sensitivity, the average increase in reaction two weeks later being 17 mm. Subsequent doses of vaccine failed to restore the sensitivity of these animals, which remained at a low plane until the test dose of virulent tubercle bacilli was given shortly after the administration of the sixth dose of vaccine, when a very appreciable increase in allergy was observed. Two assumptions can be advanced to explain the sudden change in skin sensitivity at this stage, *viz.* (*a*) a specific tuberculin response due to the establishment of the test organisms and the production of active lesions of tuberculosis, or (*b*) the entrance of the test organisms into the body and their subsequent inactivation or destruction by reason of the immunity engendered by the vaccine. An examination of the *post-mortem* records of this group of animals suggests that the second hypothesis gives the more satisfactory explanation. From Table VII it will be noticed that in five animals (215, 222, 223, 224, 232), all of which were in a

Table VII.

	Vaccinated.	
No. of Animal.	*Final Tuberculin Test. Average increase in mm.*	*Post-mortem Findings.*
215	13	—
216	12	++
220	4	++
221	11	++
222	12	—
223	11	—
224	9	—
232	12	—
	Controls.	
269	7	++++
300	27	++++

low state of allergy when the test dose was given, the tuberculin tests prior to *post-mortem* examination were strongly positive, although no lesions were found at autopsy. In the three remaining animals (216, 220, 221) in which evidence of tuberculosis was found at autopsy, the response to tuberculin was no more apparent and in one animal (220) was definitely less marked.

CHART 1.

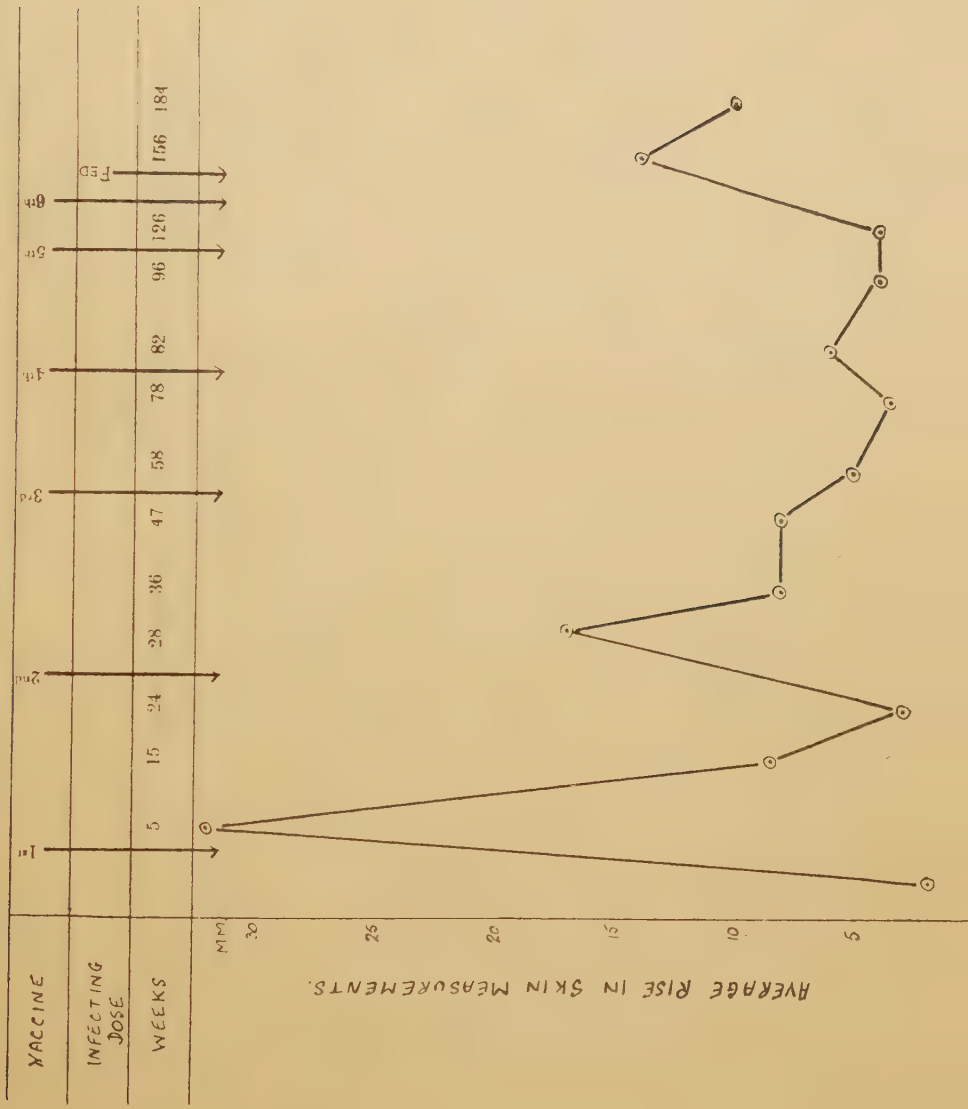
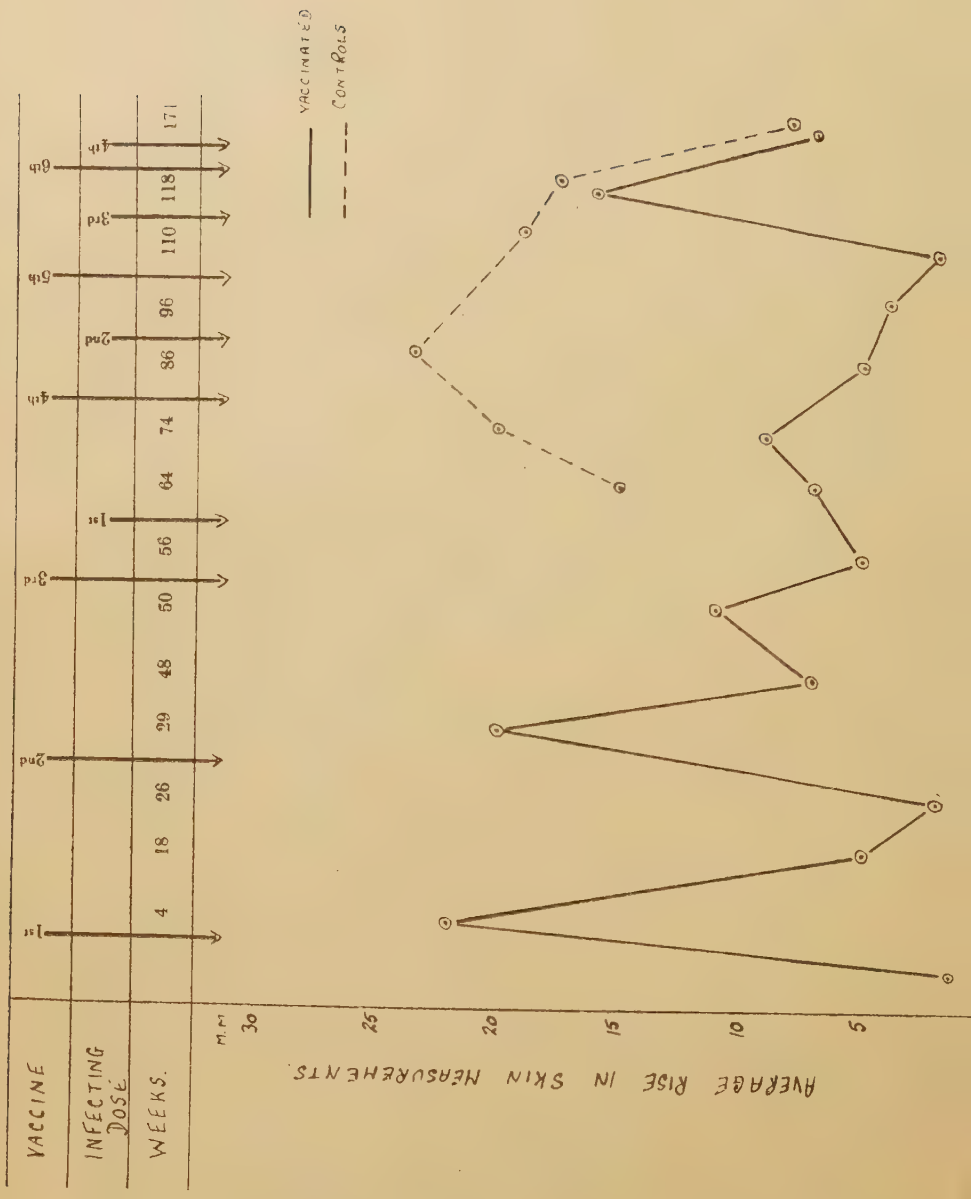

VACCINE
INFECTING DOSE
WEEKS
1st
2nd
3rd
4th
5th
6th
FED
5
15
24
28
36
47
58
78
82
96
126
156
184
30
25
20
15
10
5
AVERAGE RISE IN SKIN MEASUREMENTS.

CHART 2.


VACCINE
INFECTING DOSE
WEEKS.
1st
2nd
3rd
1st
4th
2nd
5th
3rd
6th
4th
4
18
26
29
48
50
56
64
74
86
96
110
118
171
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30
25
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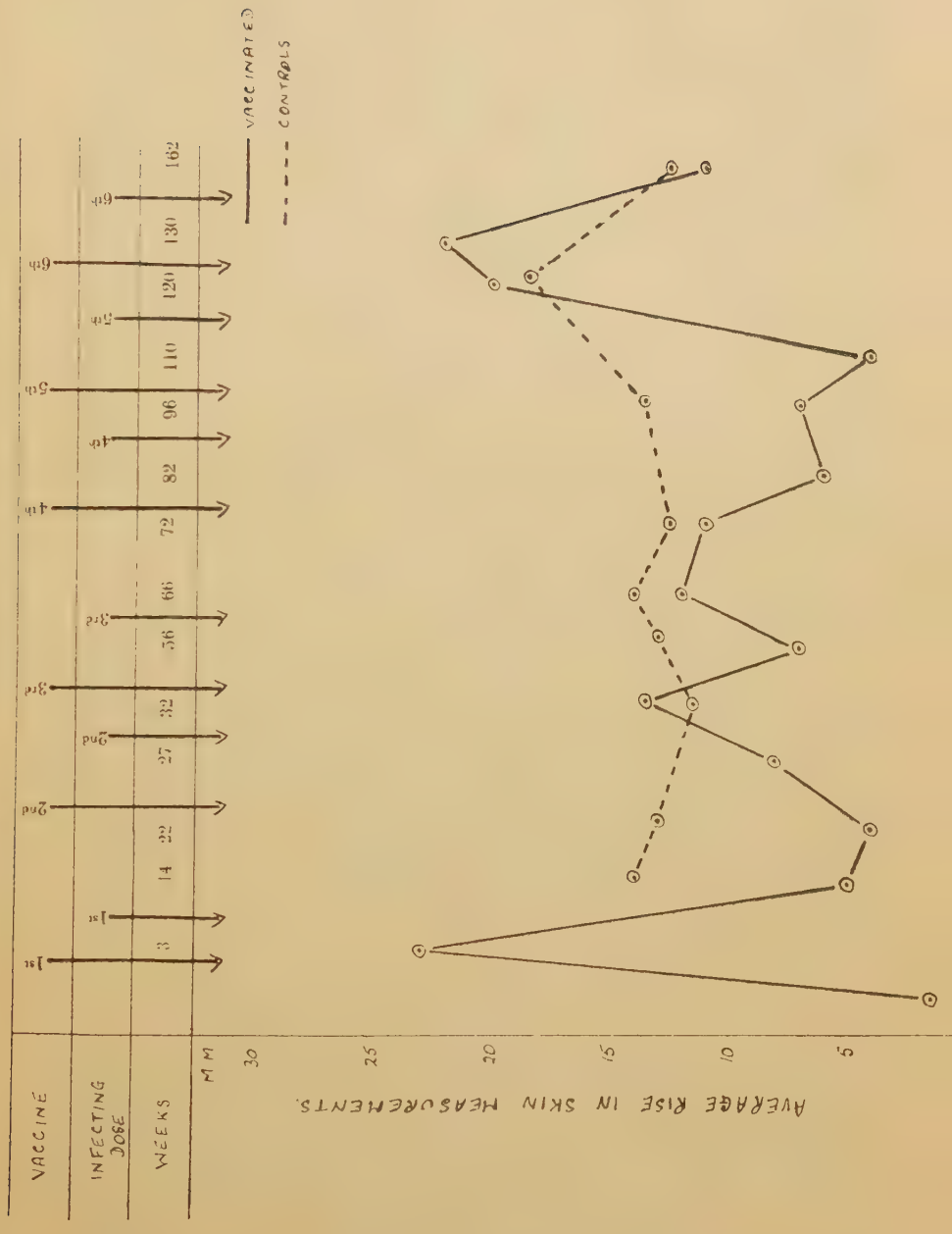
15
10
5
AVERAGE RISE IN SKIN MEASUREMENTS.
VACCINATED
CONTROLS

CHART 3.


VACCINE
INFECTING DOSE
WEEKS
MM
1st
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4th
5th
5th
6th
6th
3
14
22
27
32
36
66
72
82
96
110
120
130
162
30
25
20
15
10
5
AVERAGE RISE IN SKIN MEASUREMENTS.
VACCINATED
CONTROLS

(2) *Animals Exposed to Infection Eighteen Months after the Commencement of the Experiment.*—In the early stages of the experiment the animals in this group behaved in a manner similar to the preceding calves, since seven days after the primary dose of vaccine each one had become highly allergic, the average rise in skin measurement for the group being 22 mm. A second peak was attained after the first reinforcing dose of vaccine (20 mm.) but subsequent doses of vaccine failed to restore the sensitivity of these animals to as high a level while the decline was more abrupt. The administration of the first test dose of virulent tubercle bacilli following the third inoculation of vaccine failed to influence the general degree of allergy (compare vaccinated and control animals in Chart 2) but the sharp rise with the fifth dose of vaccine is interesting. It is possible, though unlikely, that this was due to the vaccine itself, while an alternative suggestion offers itself. Up to this period the animals had been housed mainly in pairs, but at this point they were allowed to intermingle with one another and with the controls of Group 3 (see below). There was thus a material increase in the chance of exposure to infection : it is suspected that the sharp rise in skin sensitivity is attributable to infection occurring at this stage. It is significant that in this group only one animal was completely free from tuberculosis at *post-mortem* examination.

(3) *Animals Exposed to Infection immediately after the Test Dose of Vaccine.*—Chart 3 shows the results of the serial tuberculin tests in this group. After an initial rise in the tuberculin response to a high level (23 mm.) there was a sharp drop to 5 mm. The feeding of virulent cultures was apparently without effect (compare controls and vaccinated animals in Chart 3). The second dose of vaccine induced a definite response but less marked than that resulting from the first inoculation, and subsequently the animals remained in a low state of allergy in spite of repeated doses of virulent tubercle bacilli. After the fifth dose of vaccine, however, there was a sharp rise in skin sensitivity. The explanation advanced for the similar phenomenon observed in the previous group probably holds with equal force for these animals.

Discussion.

The experiments of which the results are reported in this paper bring to a conclusion our laboratory investigations into the value of BCG vaccine as an immunising agent for cattle. The appraisement of this method of vaccination has been taken in stages, commencing with an inquiry into the most satisfactory route of administration of BCG ; the intensity of the immunity induced by a single dose of vaccine to a moderately severe infection, the experiment being of short duration ; the influence of reinforcing doses of vaccine ; and finally, the effect of repeated doses of BCG at regular intervals with

immediate or delayed exposure to infection under conditions simulating as far as possible those encountered on infected farms. It is probable that this final experiment is the most important, since it is on the ability of vaccinated cattle to withstand continued exposure to infection that the value of the product must be judged.

Few articles of importance have appeared since the publication of our last communication (Buxton, Glover and Griffith, 1934) in which the relevant literature was reviewed; moreover, in almost every instance the experiments have been conducted along lines which have differed fundamentally from the present series.

Watson (1930) described in detail the results of an enquiry undertaken by the Department of Agriculture for Canada covering a period of more than five years. The cattle vaccinated with BCG, together with suitable controls, were exposed to infection by cohabitation with tuberculous animals under natural conditions. In each case the vaccine was injected subcutaneously, the dose varying from 75 mg. to 100 mg., while the number of injections ranged from one to three at intervals of from 12 to 15 months. It is stated that at the *post-mortem* examination there was a higher incidence of tuberculosis in the vaccinated animals (73·3 per cent.) than in the controls (61·2 per cent.). Watson is of the opinion that in trials of short duration BCG appears to exert a retarding action on the development and extension of tuberculous lesions, but when the experiments are prolonged and the animals are exposed to continuous infection, the extent of the lesions in vaccinated animals is not less marked than in controls kept under similar conditions.

In a second article (Watson, 1934), he amplifies his contention that age incidence plays an important part. He asserts that whereas cattle killed one-and-a-half-years after exposure to infection showed complete or nearly complete protection (92·3 per cent. in vaccinated, as compared with 74·1 per cent. in the controls), the incidence in the two groups after an average period of exposure of four-and-a-half years was 42·1 per cent. and 56·2 per cent. respectively, *i.e.*, a figure which favoured the controls. He says " the composite picture of the BCG group may be distinguished from that of the unvaccinated group by the following features: (1) the greater degree of relative resistance at the age of puberty; (2) the failure and disappearance of that resistance at early breeding age and subsequently; (3) an accelerated activity of the disease and the preponderance of lesions of the pathologically progressive type." The investigations of Watson embrace a fairly large number of animals. Apart from the fact that he used the subcutaneous route of administering the vaccine and did not attempt to infect his animals by the direct ingestion of cultures of virulent tubercle bacilli, his experiments are in close relationship to those which are considered in this paper.

Brief reference may be made to the work of Cotton and Crawford (1932), who found that three calves vaccinated at birth by the subcutaneous route, reinoculated every year and exposed to natural

infection for two-and-a-half to three years, were as extensively affected with tuberculosis as three unprotected controls maintained under similar conditions.

Ascoli (1937) reports that five calves vaccinated three times by the subcutaneous route at the 10th, 193rd and 356th day respectively, and allowed to cohabit with tuberculous cattle 52 days after the first dose, were completely free from tuberculosis, with the exception of one which showed a single lesion in a mesenteric gland. Three controls showed slight to moderate tuberculosis.

Rankin and his co-workers (1932) vaccinated a series of calves shortly after birth with three doses of BCG by the mouth (50 mg. each dose). Six months later they were exposed to infection, together with unprotected controls, by permitting them to mix with tuberculous animals and by feeding infected milk. After six months' contact they were again placed in a tubercle-free environment. With the exception of one control which died and was free from tuberculosis, all the animals (17 vaccinated and 16 controls) were killed about two years after the commencement of the experiment. There was an increased resistance in the vaccinated calves as compared with the controls. Three vaccinated animals only were affected, the lesions being confined in each case to a single gland, while six controls showed slight lesions, which in one instance extended to the bronchial glands and the lungs. It will be noted that although the duration of the experiment was fairly lengthy, the period of exposure to infection was comparatively short, and the trivial nature of the lesions in the controls may have been attributable to this fact.

The present series differs to some extent from those of a similar nature undertaken by other workers in this field, *e.g.*, Ascoli, Watson, Rankin, etc., since the method of vaccination has been restricted exclusively to the intravenous route ; moreover, exposure to infection has been effected both by the deliberate feeding of cultures of virulent tubercle bacilli and by close cohabitation of vaccinated animals and artificially infected controls. The experiments have also furnished some information on the behaviour of cattle repeatedly vaccinated over a long period and exposed to infection at spaced intervals after the inoculation of the first dose of vaccine. One of the most striking facts has been the differences in the reactions of these groups to virulent tubercle bacilli. Thus the most favourable result has been obtained in a group of eight protected animals vaccinated at six-monthly intervals for three years and then exposed to infection by the mouth. At *post-mortem* examination six months after the infective dose six of these animals were completely free from visible lesions of tuberculosis, although in one instance viable tubercle bacilli were recovered from a gland. In the remaining two, trivial lesions were found which were in marked contrast to the extensive caseous foci which occurred in the controls. There was a striking resemblance between the extent of the protection in this group and that shown by calves which received a single dose of BCG and were tested for

immunity six months later (Griffith, Buxton and Glover, 1931). Evidently repeated inoculation of BCG vaccine at regular intervals induces an immunity which is not less potent than that resulting from a single injection. This information is of value as it gives some indication of the principles underlying vaccination with BCG. The primary injection induces the formation of innumerable minute retrogressive " tubercles " in the lungs, from which the organisms are recoverable for a period of two to four months (Buxton, Glover and Griffith, 1934). Subsequent inoculations, however, lead to a resuscitation of the original sites, but the organisms are not permitted to establish themselves in the pulmonary tissues.* The increase in resistance resulting from repeated doses does not appear therefore to be due to the creation of fresh BCG foci in the lungs, but to the stimulation of a defence mechanism which has already been evoked by the primary inoculation.

The comparatively high resistance of long duration only applies, however, to animals which have been protected from infection until the last dose of vaccine has been received. In the two groups exposed to infection immediately after primary vaccination or 18 months later the results were appreciably different. Thus, out of 15 controls, seven were severely affected, seven showed moderate or slight lesions, while one was free from macroscopical evidence of tuberculosis. Paradoxically, the animals which had been exposed to infection for the longest period were less severely affected than the others. In the corresponding group of vaccinated animals (28), ten were completely negative, fourteen showed slight or moderate lesions, while four were severely affected.

It is evident, therefore, that the length of time during which vaccinated animals are exposed to infection strongly influences the resistance which they exhibit against tuberculosis acquired by the alimentary tract. Whilst we should not go so far as to say that our results are as discouraging as those of Watson (1930) who, as a result of his experiments, found that there was no great difference in the extent of the lesions in vaccinated and unvaccinated animals, we have to admit that some of the calves were as widely affected as the controls and any resistance which was engendered in the protected animals, taken as a whole, was a matter of degree.

One of the disturbing features of this investigation has been the detection of vaccinated animals which were " carriers " of viable tubercle bacilli. In several instances a thorough search of the organs and glands failed to reveal any macroscopic evidence of tuberculosis, but suspensions of mixed glands inoculated into guinea-pigs gave rise to generalised tuberculosis. The hypothesis might be advanced that in these animals the virulent bacilli were being held in check and that in consequence the danger of spread of infection

*These observations have been confirmed in the rabbit: Innes and Glover, unpublished results.

was unlikely to occur. The evidence obtained, however, was insufficient to give an unequivocal answer to this supposition and such animals must, therefore, be regarded as under suspicion. Watson (1930) states that virulent tubercle bacilli were present in the lesion-free lymph glands of 25 per cent. of his BCG vaccinated cattle and in 8 per cent. of the controls.

The reaction of vaccinated animals to tuberculin has been considered in some detail. The practical significance of this aspect of the protection of cattle with BCG in the field is self-evident, since the tuberculin test cannot be used to differentiate the allergy dependent on vaccination with BCG from that which arises from invasion of the body with virulent tubercle bacilli. It is of some importance, therefore, to know whether the animals which are repeatedly subjected to injections of BCG remain in a state of tuberculin allergy or whether they eventually become tuberculin negative. The experiments, in so far as they go, suggest that repeated doses of BCG given by the intravenous route tend to reinforce a waning allergy but that each successive stimulus is less effective and its result is of shorter duration. By the time an animal has received five or six doses the increase in the dermal response to tuberculin is fleeting and the animal may become completely or almost completely tuberculin negative before the next injection of vaccine. This stage of partial or total anergy is not necessarily accompanied by any consistent alteration in the power of resistance to tuberculosis ; indeed, the general trend of the experiments seemed to indicate that there was no relationship between the allergy due to BCG and the resistance to experimental infection. These observations are in conformity with modern views on allergy and immunity to the effect that the extent of the resistance against tuberculosis which is exhibited by vaccinated animals is quite independent of their sensitivity to tuberculin.

It was noted in a previous article (Buxton and Griffith, 1931) that an intravenous injection of BCG (10 mg.) into a calf which was affected with spontaneous tuberculosis resulted in a severe systemic reaction. The calf in question showed at the fifteenth hour a rise in temperature to 104° F., which remained high for two days. The animal died 19 days later. As all the calves, apart from this case, used in the first and subsequent experiments were consistently tuberculin negative prior to vaccination, no untoward reactions have followed the introduction of the initial dose of BCG. A few calves died after the second or third dose with symptoms of acute anaphylaxis. In no case, however, were we able to detect lesions of spontaneous tuberculosis and it is presumed that they were sensitised to a high degree by previous injections of the vaccine. It is to be noted, however, that animals which received several doses of BCG and had been exposed simultaneously to continuous infection, rarely reacted with undue severity to the re-vaccinating doses, although it is almost certain that some of them were bearing active lesions of tuberculosis. It is possible, therefore, that whilst the

primary introduction of BCG into a tuberculous animal may occasionally result in a severe systemic shock, repeated injections of BCG may induce a state of desensitisation so that even the presence of a tuberculous focus which might be expected to render them highly sensitive, is readily tolerated. We have not observed the severe reactions reported in re-vaccinated animals by Watson (1934) which he describes as similar to an intense tuberculin reaction, but this difference may be attributable to the variation in the route of administration of the vaccine.

The experiments which are described in this paper are being supplemented by field trials in which cattle vaccinated with BCG by the intravenous route at an early age and re-vaccinated at regular intervals, have been drafted into herds which are known to be severely affected with tuberculosis. The results of continuous exposure to infection which it is suspected will be less severe than that to which the experimental animals were subjected, are not yet available.

Conclusions.

1. BCG given intravenously at six-monthly intervals in doses of 50 or 100 mg. is well tolerated by cattle.

2. Of eight calves which had been vaccinated regularly at six-monthly intervals for a period of three years, five (62 per cent.) were completely resistant to an oral administration of virulent tubercle bacilli which produced extensive local and slight generalised tuberculosis in unprotected controls. In the remaining three (38 per cent.) no macroscopic lesions were found in one, while in two the tuberculous changes were slight.

3. The resistance induced by the repeated inoculation of BCG over a period of three years was overcome by continued exposure to infection immediately after the first dose of vaccine or after a lapse of 18 months. In 28 animals, ten were completely free from tuberculosis (35·5 per cent.), 14 showed slight or moderate lesions (50 per cent.), while four were severely affected (14·5 per cent.).

4. Distinct fluctuations in the sensitivity to tuberculin were observed during the course of vaccination with BCG. In general, each reinforcing dose tended to augment the skin sensitivity, but the stimulus was less apparent with successive doses of the vaccine.

5. In a proportion of vaccinated animals, which showed no visible lesions at *post-mortem*, virulent organisms were obtained from various lymphatic glands by guinea-pig inoculation.

Acknowledgment.

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THE FATE OF COPPER IN THE BLOOD STREAM

By Alfred Eden
and
H. H. Green,
The Veterinary Laboratory, Ministry of Agriculture, Weybridge.

Few studies have been made on the fate of copper injected into the blood stream of animals. Lander (1926) states that the injection of non-irritant copper salts—double alkali tartrates and albuminates—induces slow and weak locomotion and later paralysis in which the heart and respiration are involved. If the animal survives, loss of flesh, inappetence, albuminuria, icterus, anæmia and diarrhœa may ensue. Cushny (1932) records that the intravenous injection of copper causes similar initial locomotor effects followed later by complete paralysis. Respiration ceases before heart-beat and an initial rise in blood pressure is followed by a fall due to heart weakness and dilatation of the blood vessels. Fatty degeneration of the liver, kidney and heart, sometimes accompanied by icterus, anæmia, hyperæmia along the intestine and in the kidney, have been recorded.

Very few studies of the blood copper level following injection and ingestion have been reported, since accurate estimation of blood copper has been feasible only within the last few years. The present paper deals with experiments along these lines using the rabbit as pilot animal and sheep where larger quantities of blood and plasma were required.

Copper estimations were made by a rapid and accurate modification of the sodium diethyldithiocarbamate method in which destruction or organic matter was effected by the use of a wet ashing digestion mixture of sulphuric, perchloric and nitric acids.

Distribution of Copper between Cells and Plasma.

Elvehjem (1935) in commenting on the distribution of normal blood copper between cells and plasma mentions that he and co-workers (1929) found the major portion in the corpuscular fraction, and Sarata (1933) confirmed this. Schindel (1931) and Guillemet (1932) however had reported more copper in the plasma than in the cells, whilst Tompsett (1934) found the blood copper to be approximately equally distributed over each. Difficulties with earlier methods may have been responsible for the lack of decisive information on this point but with the present method the findings of Tompsett have been confirmed. Blood from sheep and cattle was taken into copper-free potassium oxalate (2 mg. per ml.) and the plasma obtained by centrifuging. Blood from sheep was also allowed to clot and the serum copper values determined. As shown in Table I values for plasma or serum and for the corresponding whole blood were found to be approximately the same.

TABLE I.

COPPER CONTENT OF WHOLE BLOOD, PLASMA AND SERUM.

Copper in mg./100 ml.

Animals.	*No.*	*Whole Blood.*	*Plasma.*	*Animals.*	*No.*	*Whole Blood.*	*Plasma.*
Cattle	1	0·080	0·080	Sheep	5	0·078	0·081
	2	0·076	0·080		6	0·123	0·121
	3	0·070	0·069			*Whole Blood.*	*Serum.*
	4	0·087	0·090				
	5	0·071	0·068		7	0·142	0·149
	6	0·096	0·082		8	0·134	0·136
Sheep	1	0·122	0·116		9	0·130	0·128
	2	0·156	0·149		10	0·136	0·140
	3	0·138	0·133		11	0·120	0·120
	4	0·128	0·125				

RATE OF FALL OF BLOOD COPPER AFTER INTRAVENOUS INJECTION.

(*a*) *Rabbits.*

Preliminary investigations into the rate of fall of blood copper following intravenous injection of copper salts were made upon rabbits. Oxalated blood samples were taken from the posterior auricular vein of one ear and the injection made into the corresponding vein of the other ear. The copper salt employed in all cases was the sulphate, although inflammation of the ear vein invariably accompanied the injection and complete localised necrosis followed any accidental slipping of the needle. The use of the sulphate in place of less irritant double salts, however, ensured that the copper was injected in a highly ionised state.

The minimum lethal dose of copper by intravenous injection was found to be about 5 mg. for a rabbit of 2 kg. body weight. 10 mg. were fatal within a few minutes. A safe amount of copper appeared to be about 2 mg. per kg. body weight for a rabbit, this figure agreeing with the finding of Bjerrum and Henriques (1935).

TABLE II.

RABBITS INJECTED WITH 3 MG. COPPER CONTAINED IN 0·6 ML. COPPER SULPHATE SOLUTION.

Copper in mg./100 ml. blood.

Ref. No.	4152	4153	14
Initial value	0·097	0·087	0·098
5 mins. after injection	0·918	0·680	0·949
60 „ „ „	0·378	0·311	0·544
2 hours „ „	—	—	0·279
3 „ „ „	—	0·156	—
4 „ „ „	—	—	0·152
5 „ „ „	—	—	0·135
6 „ „ „	0·126	—	—
7 „ „ „	—	—	0·121
24 „ „ „	0·100	0·085	0·099

The normal blood copper content of the laboratory rabbits used lay between 0·08 and 0·10 mg. per 100 ml. Three rabbits were injected with 3 mg. copper and the blood copper values were determined at known intervals of time after injection. These data are given in Table II.

The blood copper in two of the three rabbits rose to about ten times the initial value after the injections. The first bleeding was made after about five minutes, during which time the injected copper had thoroughly mixed with the circulating blood. The rate of fall was most rapid over the first hour and by the end of the third or fourth hour the blood copper was already less than double the normal value. Afterwards the fall was much more gradual, normality, however, being regained within 24 hours, indeed almost within six or seven hours. These results are expressed graphically in Fig. 1.

Two rabbits were then injected intravenously with 5 mg. of copper as the sulphate, this dose being on the borderline of toxicity. The results given in Table III indicate that the general findings with this higher quantity are the same as shown with the 3 mg. quantity. The fall during the first hour is very rapid and the normal level is practically regained within about six hours. The same results were obtained when only 1 mg. of copper was injected although naturally the initial rise was not so pronounced.

TABLE III.

RATE OF FALL OF BLOOD COPPER FOLLOWING INTRAVENOUS INJECTION OF COPPER.

Copper in mg./100 ml. blood.

	Copper Injected.			
	5 mg.	*5 mg.*	*1 mg.*	*1 mg.*
Ref. No.	*X*	4187	4193	4277
Initial value	0·098	0·087	0·089	0·094
5 mins. after injection	1·176	0·767	0·575	0·485
60 „ „ „	0·647	0·444	0·226	0·214
2 hours „ „	—	0·229	—	0·146
3 „ „ „	—	—	0·175	—
4 „ „ „	0·179	0·118	—	0·114
6 „ „ „	0·126	0·099	0·105	0·099
24 „ „ „	0·096	0·098	0·090	0·099

This tendency of blood to regain its normal physiological level with great rapidity in respect to injected substances is, of course, not confined to copper. Green obtained similar results for arsenic (1918) and numerous investigators have reported comparable data for such ions as calcium and magnesium. The curves obtained by plotting the values against time after injection are logarithmic, the rate of fall at any given moment being roughly proportional to the excess above the normal value. Although it is well known that copper readily forms complexes with protein, the present data indicate that if such

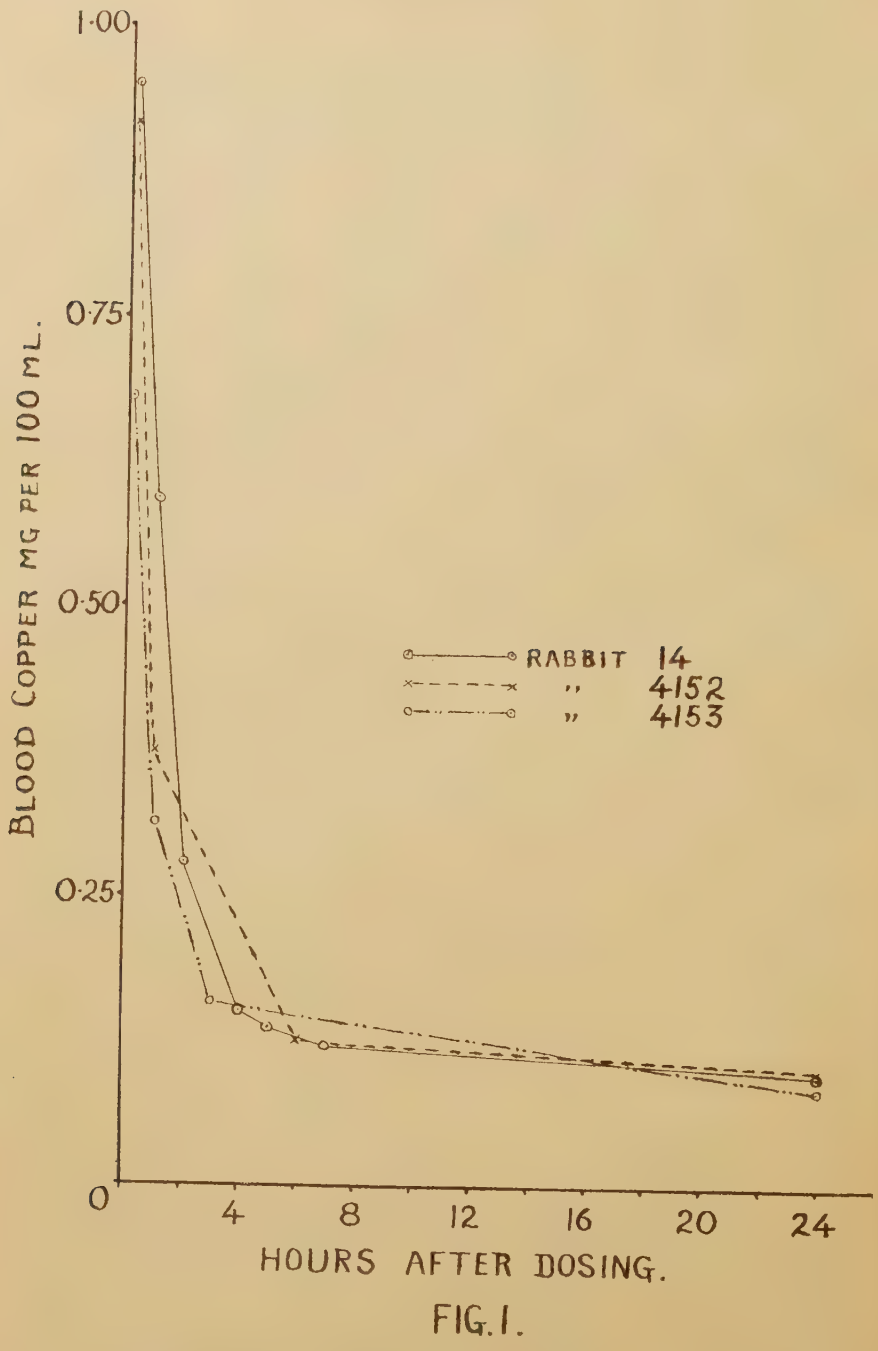

FIG. 1.—Blood Copper Values of Rabbits following intravenous injection of 3 mg. copper.

compounds are formed between blood proteins and injected copper the combination must be a loose one, since the blood value falls to normal so rapidly by temporary distribution over the tissues and slow subsequent elimination.

(b) Sheep.

The equal distribution of copper between plasma and corpuscles has already been confirmed. Bjerrum and Henriques (1935) studied the distribution of the element in rabbits following intravenous injection, using a variation of Warburg's cysteine-oxidation method with arbitrary correction factors, but since the absolute volume of blood in the rabbit is small and there are considerable difficulties in obtaining repeatedly from the ear sufficient blood for analysis by the present method, larger animals were used for the following experiments.

As a preliminary, the response of sheep's blood to injected copper was compared with that of the rabbit. Two sheep, Nos. 832 and 836, were injected intrajugularly with 50 ml. copper sulphate solution containing 100 mg. copper, equivalent to about 2 mg. per kg. body weight. The animals appeared quite normal after the injections and bleedings were made at known intervals but Sheep 832 showed symptoms of distress four hours afterwards, including gasping and laboured breathing. The venous return was very sluggish and a blood sample was secured with difficulty. The animal died an hour later and samples of its tissues were taken for analysis. These and other data will be reported upon in a further communication. The blood data are given in Table IV.

TABLE IV.

INTRAVENOUS INJECTION OF SHEEP WITH 100 MG. COPPER IN 50 ML. COPPER SULPHATE SOLUTION.

Copper in mg./100 ml. blood.

	No. 836	*No.* 832		*No.* 836	*No.* 832
Initial value ...	0·152	0·142	2 hours after injection	0·213	0·97
5 mins. after injection	0·730	2·01	4 „ „ „	0·169	0·67
30 „ „ „	0·420	1·65	6 „ „ „	0·168	—
60 „ „ „	0·238	1·18	24 „ „ „	0·170	—

The rapid rate of fall of blood copper over the first hour is again noteworthy, the general behaviour being similar to that of the rabbits. The final value in Sheep 836, however, did not quite reach the initial value but remained some 12 per cent. higher, 24 hours after injection. The low value of No. 836 compared with No. 832 five minutes after the copper injection is to be noted and suggests that the needle may have slipped out of the vein during injection although this was not observed to have happened. The fall over the first hour in No. 832 was very rapid but thereafter was not so pronounced and the amount

of copper injected was such that toxic symptoms supervened. *Post-mortem* examination revealed no macroscopic lesions and the immediate physiological cause of death could not be deduced.

Since the actual quantity of copper reaching the circulation was uncertain with Sheep 836 a third experiment was undertaken ; on this occasion, plasma copper was determined as well as that in whole blood. Sheep 835 was given 80 mg. of copper as the sulphate in 40 ml. solution intravenously and the animal appeared to be in no way affected by the injection. Periodic bleedings were made into oxalate, and after portions had been removed for copper determinations on whole blood the remainder was immediately centrifuged and the plasma recovered for analysis. The next day the sheep was noticed to be breathing rapidly and passing copious watery fæces. The urine was deep brown in colour but was not clinically examined. Cotton cake and hay were fed to correct the diarrhœa which ceased after two days although the fæces remained soft. The urine appeared more normal but the animal was drinking very copiously. The earlier samples of plasma were slightly hæmolysed but later became more normal. On the fifth day the sheep showed marked symptoms of distress, bleated repeatedly and was unable to rise. It died in the afternoon. Apart from a slight congestion of heart and lungs there was no gross abnormality on *post-mortem* examination. In Table V are recorded the whole blood and corresponding plasma copper values. The calculated figures in the third column are obtained by taking the corpuscular volume as 38 per cent. of the blood volume. The corpuscular copper is thus found to be $\frac{1}{38}$ (100 $\times$ whole blood value minus 62 $\times$ plasma copper value). Unfortunately such a method of calculation, involving a multiplication of a summation of analytical errors, can throw the whole enlarged error on to the calculated corpuscular value. Thus, admitting a real error of $\pm$ 5 per cent. on each analytical determination, *i.e.* $\pm$ 0·1 mg. on real values of 2·0 mg. per cent. for both plasma and whole blood values, the method of calculation makes this error $\frac{1}{38}$ (100 $\times$ ($\pm$ 0·1) — 62 $\times$ ($\pm$ 0·1), an overall error $\pm$ 0·43 giving a calculated value ranging from 1·57 to 2·43 mg. per cent. Hence, in column (3) of Table V the possible range is given taking into account the permissible $\pm$ 5 per cent. error in analysis, as well as the mean value. The data are expressed to two decimal places, from figures calculated to three places.

Table V shows the high values reached following the injection, about 15 times normal in whole blood and over 20 times in the plasma, corresponding roughly to the theoretical elevation calculable from the probable blood volume of the sheep in relation to the dosage injected. The initial rate of fall was again very rapid, especially so in the plasma, although the figure on the latter was still above that of the whole blood an hour afterwards. By the end of the second hour the plasma and whole blood values were much the same, indicating

TABLE V.

INTRAVENOUS INJECTION OF SHEEP 835 WITH 80 MG. COPPER.

	Whole Blood.	*Plasma.*	*Calculated Corpuscular Values.*	
			Range.	*Mean.*
Initial value	0·15	0·15	0·12 to 0·18	0·15
5 mins. after injection	2·38	3·68	(— 0·37 „ 0·86)	0·25
30 „ „ „	1·80	2·05	0·97 „ 1·78	1·38
60 „ „ „	1·36	1·57	0·72 „ 1·33	1·03
1½ hours „ „	1·19	1·22	0·88 „ 1·40	1·14
2 „ „ „	1·14	1·13	0·91 „ 1·39	1·15
3 „ „ „	0·97	0·96	0·78 „ 1·18	0·98
5 „ „ „	0·71	0·34	1·20 „ 1·44	1·32
7 „ „ „	0·46	—*	—	—
24 „ „ „	0·27	0·21	0·32 „ 0·42	0·37
48 „ „ „	0·26	0·22	0·27 „ 0·37	0·32
96 „ „ „	0·23	0·23	0·19 „ 0·29	0·24

* Plasma sample lost in centrifuging.

that approximate copper equilibrium had been established between cells and plasma. The figures for the possible range of corpuscular copper values, with their means, show that five minutes after injection probably very little copper had penetrated the corpuscles but that after 30 minutes the corpuscular copper had increased to between about 1 and 1·75 mg. per cent. and values of the order of 1 mg. per cent., or seven times the normal value, were sustained over at least five hours. The values for whole blood and plasma after three hours were falling at the same rate. By the end of the fifth hour the plasma copper was less than half that of the whole blood, indicating that the greater part of the residual copper was now lodged in the corpuscles. Unfortunately the plasma sample from the seventh hour was lost in centrifuging. Even after 24 hours the whole blood figure was still above that of the plasma and each was still above normal. This supranormal value persisted until death although by this time cells and plasma had once again come into copper equilibrium. Owing to the sluggish venous return it was impossible to secure a blood sample on the day the animal died.

Thus, although the actual corpuscular figures cannot be deduced with any high degree of accuracy, the plasma and whole blood figures, accurate to within ± 5 per cent., show that copper, penetrating into or adsorbed upon the corpuscles, appears to be more tenaciously retained; by the fifth hour, although the plasma figure was only about twice the normal value, the corpuscular figure was about four times the plasma figure (between 1·2 and 1·44 mg. per cent.) or nine times normal. These results indicate either a slow permeability of the cell to copper or at least temporary adsorption on the membrane.

The same type of experiment was repeated on Sheep 831 into which, however, a much lower quantity of copper was injected. 30 mg. copper as the sulphate were made up in 0·9 per cent. saline

and slowly injected intravenously, the slight degree of hæmolysis noted in the plasma of the previous sheep thus being prevented. The results are given in Table VI.

TABLE VI.

INTRAVENOUS INJECTION OF SHEEP 831 WITH 30 MG. COPPER (AS THE SULPHATE). Copper in mg./100 ml.

	Whole Blood.	*Plasma.*		*Whole Blood.*	*Plasma.*
Initial value ...	0·131	0·131	4 hours after injection	0·154	0·154
5 mins. after injection	0·787	1·060	5 „ „ „	0·151	0·148
60 „ „ „	0·218	0·217	24 „ „ „	0·149	0·148
2 hours „ „	0·175	0·175			

As before the initial rise was most marked in the plasma but after an hour the values for whole blood and plasma were the same, indicating that in the process of elimination from the blood stream the greater part of the copper had been removed before extensive penetration of the corpuscles had occurred. With the smaller quantity (about 0·6 mg. per kg. body weight) the initial rise was only about one-third that encountered in the previous experiment which had terminated fatally and the rate of fall was so rapid that within an hour the figure was no more than one and a half times the initial value. Within this period penetration of corpuscles was just sufficient to bring them into equilibrium with plasma. The initial value was not quite reached within 24 hours, both corpuscles and plasma figures then being still 10 per cent. above normal.

Bjerrum and Henriques (1935) performed a similar series of injections with rabbits at the higher level of 2 mg. per kg. body weight, giving hæmatocrite values and expressing their results as mg. of copper per litre of plasma and of cells. Some of their figures have been recalculated back to the basis of whole blood, expressed as mg. per cent., for comparison with the present experimental data. The recalculated figures are given in Table VII.

The general inferences to be drawn from these data are very similar to those obtained from the present series of experiments. Following injection the plasma values rose considerably above those for whole blood but after an hour the values were much the same, indicating that copper had penetrated the corpuscular membrane or had been adsorbed upon it. From this point the plasma values fell more rapidly than the whole blood values, showing that the copper was held inside or upon the membrane rather more tenaciously. At any rate diffusion of copper through the membrane of the red blood cells, if it occurs, is not a very rapid process, as would be expected from the known general impermeability of the membrane to cations. The initial levels, however, in the experiments of Bjerrum and Henriques were not approached so rapidly as in the majority of the present findings, probably because of the high initial dosage.

Table VII.

Data of Bjerrum and Henriques, Recalculated for Whole Blood and for Plasma. Copper in mg./100 ml.

	Rabbit 1.		*Rabbit* 2.	
	Whole Blood.	*Plasma.*	*Whole Blood.*	*Plasma.*
Initial value	0·092	0·082	0·073	0·067
Immediately after injection	2·49	3·45	1·88	2·41
10 mins. after injection	1·96	2·56	—	—
30 „ „ „	1·70	2·14	—	1·67
60 „ „ „	1·23	1·16	1·03	1·15
120 „ „ „	0·87	0·75	—	0·84
180 „ „ „	0·68	0·52	0·423	0·329
280 „ „ „	—	—	0·271	0·198
1,230 „ „ „	—	—	0·153	0·104

Rabbit 3.

	Whole Blood.	*Plasma.*
Initial value	0·059	0·037
Immediately after injection ...	1·65	2·54
35 mins. after injection ...	1·56	1·82
95 „ „ „	0·806	0·780
185 „ „ „	0·455	0·318
365 „ „ „ ...	0·231	0·152
1,440 „ „ „ ...	0·112	0·076

Effect of Oral Dosing upon Blood Copper Level.

There are very few references in the literature to the effect of oral dosings with copper upon the blood copper picture. As is well known, copper sulphate administered *per os* acts as a powerful emetic upon man and the dog, but in rabbits and sheep, which apparently lack a well developed vomiting centre, copper administered orally passes wholly through the digestive tract. The effect of concentrated solutions is to cause anorexia, gastro-enteritis, colic and diarrhœa.

In the first experiment 20 ml. of a solution containing 200 mg. copper were given by stomach tube to a rabbit. The animal manifested no immediate ill effects but within a short time showed symptoms of slight convulsions, resembling hiccoughs. A blood sample taken after two hours was found to contain the high figure of 3·57 mg. copper per 100 ml., pointing to rapid absorption of the metal from the intestine when administered in dilute solution. After four to five hours the animal died and *post-mortem* examination revealed acute gastritis with desquamation of mucosa. A repetition of this experiment was made upon a rabbit from which blood samples were taken at shorter intervals. The animal showed signs of increased respiration and within an hour, collapsed and rolled over on its side, from which position it could not raise itself on stimulation. Death occurred in just over two hours after dosing and although an attempt was made to secure a blood sample

at death by immediately cutting the jugular vein, the blood was found to have clotted. The blood copper figures were :—

After 30 minutes 1·38 mg./100 ml.
After 60 minutes 3·23 mg./100 ml.

Of four rabbits given 100 mg. copper in solution by stomach tube two died and the other two survived showing no obvious ill effects. The blood copper picture of the two fatal cases is recorded in Table VIII.

TABLE VIII.

RABBITS GIVEN 100 MG. COPPER BY STOMACH TUBE WITH FATAL SEQUENCE.
Copper in mg./100 ml. blood.

	No. 4278	*No.* 12		*No.* 4278	*No.* 12
Initial value ...	—	0·092	2 hours after dosing	2·033	0·985
30 mins. after dosing	0·441	0·220	3 „ „ „	3·540*	1·657
60 „ „ „	0·748	0·412			

* *Post-mortem* sample.

Rabbit 4278 died about three hours after dosing and a blood sample was secured from the heart shortly after death. Rabbit 12 showed no symptoms during the first three hours but after five hours it was impossible to obtain blood from the ear veins ; the animal died after six hours. As shown in Table VIII there was progressive absorption of copper into the blood and although, as already demonstrated, excess is rapidly eliminated from the circulation, the rate of accumulation was greater than that of elimination. It is important to note that this progressive rise of blood copper is characteristic of dosing by stomach tube and presumable flushing of the duodenum. As will be reported later larger quantities of copper have been ingested daily by rabbits after drying the solution on to food, without any appreciable rise in the blood copper content even after long periods of feeding.

The data from the two non-fatal cases of dosing with 100 mg. copper by stomach tube are recorded in Table IX and Fig. 2.

TABLE IX.

RABBITS GIVEN 100 MG. COPPER BY STOMACH TUBE WITH NON-FATAL SEQUENCE.
Copper in mg./100 ml. blood.

	No. 4186	*No.* 14		*No.* 4186	*No.* 14
Initial value ...	0·104	0·098	5 hours after dosing	0·480	0·177
30 mins. after dosing	0·346	0·166	6 „ „ „	0·183	0·134
60 „ „ „	0·470	0·260	7 „ „ „	0·151	0·117
2 hours „ „	0·954	0·434	24 „ „ „	0·096	0·108
3 „ „ „	0·877	0·459			

Fig. 2 shows graphically the rapid absorption of dissolved copper into the blood stream, followed by the normal rate of fall. By interpolation the points of maximum blood copper are found, about 1·06 mg.

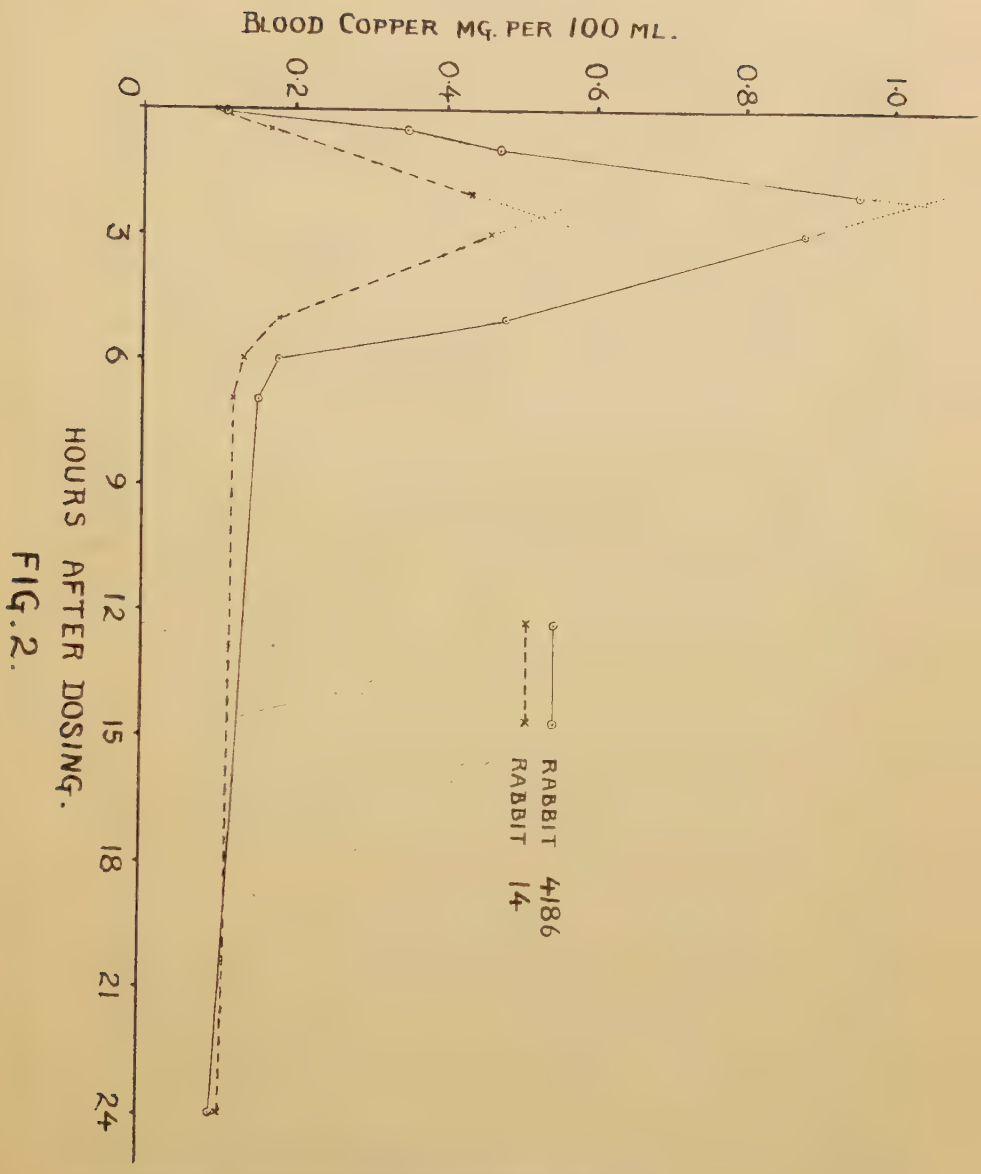

FIG. 2.—Blood Copper Values of Rabbits following dosing by stomach tube of 100 mg. copper as the sulphate.

per cent. from No. 4186 and 0·52 per cent. for No. 14. The difference in the two maxima reached depends upon relative rates of absorption from the intestine and distribution from blood to tissues and may also well depend upon the extent to which the proteins of the food and of the mucosa are able to adsorb or combine with copper salts under varying *p*H conditions. That wide variations in maxima do

occur under the same conditions of dosing of rabbits of about the same live weight is, however, interesting in itself. In each of the non-fatal cases the maximum figure was reached about two and a half hours after dosing but in the two fatal cases (Table VIII) the blood copper was still rising rapidly to still higher values at the end of the third hour.

The physiological mechanisms through which copper exerts its toxic action are not clear. Strong solutions given *per os* cause a marked gastro-enteritis and much smaller quantities injected directly into the blood stream may cause death within a short time with symptoms of paralysis and gradual respiratory failure. Toxic effects may also be cumulative and associated with pronounced liver lesions, as in cases of death from chronic copper poisoning induced by the continuous feeding of non-toxic quantities of copper over a period of weeks or even months.

Reviewing the experiments presented so far on fatal and non-fatal effects from both intravenous and oral injections, the maximum blood copper figures expressed as mg. per cent. are :—

	Fatal Cases.	*Non-Fatal Cases.*
Sheep ...	2·01, 2·38	0·73, 0·79
Rabbits ...	3·57, 3·23, 1·66, 3·54	0·68, 0·92, 0·95, 0·49, 1·06, 0·52, 1·18, 0·78, 0·58

It is impossible from such limited data to generalise on what constitutes a fatal level of copper in the blood, but the upper limit for blood copper not attended by fatal consequences seems to lie between 1·2 and 1·6 mg. per cent. for rabbit, with values of the same order or possibly lower for the sheep.

(*b*) *Sheep.*

Copper sulphate has a widespread use as a therapeutic agent against certain worms of the fourth stomach in sheep, in particular *Haemonchus contortus*. For this purpose a solution of copper sulphate is employed, alone, in conjunction with nicotine, or with sodium arsenite as in South Africa under the name of " wire-worm remedy." The normal oral dose for an adult sheep as recommended by McEwen (1935) is 100 ml. of a 1 per cent. solution, equivalent to 1 gramme of copper sulphate or about 0·25 gramme copper. This dose is generally considered effective in action as well as safe and it was considered of interest to follow the blood picture of a sheep on this dosage. These data are presented in Table X.

TABLE X.

SHEEP 833 : ORAL DOSAGE WITH 100 ML. 1 PER CENT. COPPER SULPHATE SOLUTION. Copper in mg./100 ml. blood.

Initial value	0·138	2 hours after dosing ...	0·132
40 mins. after dosing ...	0·149	3½ „ „ „ ...	0·134
75 „ „ „ ...	0·140	4½ „ „ „ ...	0·134

It will be noted that there was no perceptible change in the blood copper content, within the limits of experimental error, following dosing with the usual therapeutic quantity of copper sulphate. In Table XI are given the results following oral administration of double the therapeutic dose.

TABLE XI.

SHEEP 750: ORAL DOSAGE WITH 100 ML. 2 PER CENT. COPPER SULPHATE SOLUTION. Copper in mg./100 ml. blood.

Initial value	0·122	5 hours after dosage ...	0·136
60 mins. after dosing ...	0·150	7 „ „ „ ...	0·129
2 hours „ „ ...	0·149	24 „ „ „ ...	0·130
3 „ „ „ ...	0·136		

Beyond a slight rise within the first two hours the blood copper values did not deviate significantly from the initial value. These figures are values for jugular blood samples and it is possible that a more significant rise occurred in the portion of the circulation from the gut to the liver, the hepatic portal circulation. Owing to its anatomical position the liver receives blood directly from the absorbing areas of the small intestine and by virtue of the liver's capacity for storage blood reaching the heart from the liver might possibly be somewhat lower in copper than that reaching the liver from the intestine. The assumption that there was a wide difference in copper content of the blood between the hepatic portal and the general circulation would, however, postulate a very rapid and efficient absorption of excess copper by the liver from the blood and it seems more logical to assume that the jugular vein sample is of the same order as that obtaining in any other part of the blood circulation.

In considering the action of copper sulphate on certain parasites of the alimentary tract, the *modus operandi* of parasiticidal effect is unknown. Most of these parasites lie buried in the mucosa of the tract and obtain their food by direct access to the blood vessels supplying the tract of the host. Copper acting upon these forms might theoretically do so by direct penetration of their cuticle or through the blood stream of the host or *via* the point of insertion of the buccal lancets of the parasite through the mucosa. *Hæmonchus contortus* against which copper sulphate has an almost specific action is said by some helminthologists to be affected by the copper because it is such a voracious blood sucker and that possibly the copper acts through the blood supply to the abomasum where the worm is found.

The absence of rise in blood copper after therapeutic dosing with copper sulphate, here recorded, is against any such view, and in any case one would be inclined to assume that a level of blood copper which did not affect the host would not affect the parasite. In this connection it may be mentioned that a tenfold variation in blood copper content was found among some 300 Northumbrian ewes (Eden, 1939) which nevertheless did not show any significant differences in regard to resistance to parasitic infestation. The concentra-

tion of copper in the actual abomasal contents is enormously higher, probably 50 to 100 mg. per cent. for some time after dosing and a portion is visibly adsorbed on the mucosa, probably in loose combination with mucin. It therefore seems more probable that the damage to the worm is either a contact effect or a transference of copper in high concentration along the channel of penetration of sucking parts into the capillaries. The supposed anticoagulant (Mönnig) and presumably vaso-dilator secretion of the mouth parts may possibly facilitate local solution of copper adsorbed by the mucosa.

Summary.

(1) The minimum lethal dose of copper, intravenously injected as the sulphate, is about 1·5 mg. per kg. body weight for the rabbit and below 2 mg. for the sheep. Administered in solution by stomach tube 50 mg. per kg. is a borderline toxic dose for the rabbit, although higher quantities can be administered daily for long periods if previously dried on food.

(2) After intravenous injection of copper sulphate into rabbits and sheep the immediate rise of blood copper is followed by rapid distribution over the tissues and almost normal values are approached within five hours of a non-fatal injection. Graphs expressing blood copper values in relation to time after injection are logarithmic, indicating that the rate of disappearance is proportional to the excess above normal at any given moment.

(3) The copper content of cells and plasma in normal blood of sheep, and also of cattle, is approximately the same. After enforced elevation of blood copper by intravenous injection of the sulphate the plasma level is at first higher than that of whole blood and the rate of disappearance from plasma is also higher. After equilibrium is established between corpuscles and plasma the rate of disappearance is much the same from both. With large doses, however, raising blood copper to very high levels, the rate of fall for plasma is at first faster and thereafter slower than for whole blood. Copper appears to penetrate the corpuscles or be adsorbed on the corpuscular membrane and thereafter to be somewhat tenaciously retained.

(4) When high doses of copper sulphate, toxic or sub-toxic, are given in solution by stomach tube the blood copper value rises to levels representing the balance between rate of absorption into the blood and rate of disappearance from it, relatively high maxima being reached within a few hours. No significant rise occurs if the solution is previously dried on food. In cases of acute poisoning after oral administration the physiological effects of a high blood copper level are superimposed upon those of a gastro-enteritis.

(5) The upper level of blood copper not attended by fatal consequences would appear to lie between 1·2 and 1·6 mg. per cent., or about ten times the normal, for the rabbit, and be somewhat lower for the sheep.

(6) There was no appreciable rise in blood copper of sheep dosed orally with the anthelmintic dose of 1 gm. copper sulphate in 100 c.c. solution and only a slight rise associated with twice this amount.

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TREATMENT OF *TRYPANOSOMA BRUCEI* INFECTION IN HORSES

By J. L. Stewart, M.C., B.Sc., M.R.C.V.S.,
Director of Veterinary Services, Northern Territories, Gold Coast.

There is so little literature on the subject of *T. brucei* infections of horses that the following case notes, though covering three animals only, may be of interest. In the Gold Coast, *T. brucei* infection in horses is virulent and recoveries are very rare. The writer had never treated a case successfully until the instances now quoted.

Case No. 1.—A horse infected in August, 1938, was treated with a combination of naganol, two doses of 2 grammes and one of 4 grammes, at weekly intervals as recommended by the manufacturers, interspersed with tartar emetic dosage from 12 to 15 grains : novarsenobillon was also given as a tonic. After the third dose of naganol, trypanosomes appeared again in the blood six days later ; treatment was recommenced giving 4 gramme doses, after the second of which alarming symptoms appeared ; the testicles were enlarged, the anus was ulcerated (this is a common toxic symptom of naganol) and six days after the second dose the horse developed an acute coronitis followed by laminitis. The animal was very lame and in great pain for several days : sepsis appeared under the coronary band and in the foot. However, the feet gradually recovered under treatment but the horse again relapsed to *T. brucei* : antimosan combined with N.A.B. was substituted for the previous treatment and the trypanosomes disappeared from the blood ; after two doses of antimosan naganol was again given but in 2 gramme doses only, which treatment was carried on for five weeks when the weekly dose was increased to 3 grammes for seven weeks, the naganol always being reinforced by a dose of 40 c.c. antimosan on the same day. No adverse toxic symptoms occurred during this phase save slight swelling of the sheath. Then a dose of 2·5 grammes of antrypol was substituted for the naganol, which caused tenderness of all four feet, but that disappeared rapidly. Treatment was then stopped to see what would happen and since then, the end of January, 1939, five months ago, no trypanosomes have been found in the blood and the horse has been gaining weight ever since. He has been exercised fairly severely and appears to be recovered but will be kept under observation.

Case No. 2.—This horse became infected in early December and was put on the combined antimosan and naganol treatment which was the second type of treatment which the first horse received. He was ten weeks on this treatment with the addition of N.A.B. but received 2 gramme doses of naganol only, no more. During this period he showed no parasites in the blood. Then on the arrival of antrypol from Britain, which had been recommended by a Medical Officer as being less toxic than naganol, the horse was put on 3 grammes of antrypol : after the second weekly injection of antrypol,

the animal began to show the same toxic symptoms as No. 1 horse showed after his second course of naganol. His feet were worse than horse No. 1 and he was in such pain one day that destruction was contemplated but he became rather better the next day. No further treatment was given, the horse slowly recovered from the toxic symptoms of antrypol and has shown no further trypanosomes in his blood and looks like a recovery. He has been working in a cart for two months.

Case No. 3.—The third horse received a course of three injections of antrypol and tartar emetic. After the third dose, he showed œdema of the chest, which extended to the neck and throat. He became so weak that he was destroyed. *Post-mortem* lesions were indefinite and it is not clear whether death was due to the disease or to the treatment but this horse was in a very poor state when he arrived at the laboratory and had been infected for some time, the owner attempting treatment himself, of the wrong type.

If the first two cases are recoveries and they appear to be, they are the first from *T. brucei* in the Gold Coast. This parasite is very fatal to horses, which appear to be very susceptible to naganol and antrypol, but both drugs are tolerated very well by human beings, who can easily stand half the horse dose. Little can be found in literature about treatment of equine *T. brucei*, which is why the above treatment was so experimental and that it was so opportunistic was due to the sudden appearance of toxic symptoms. It is proposed to treat future cases with the combined antimosan and naganol treatment, giving very small doses of naganol.

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INFECTION OF A BOVINE BY HUMAN TYPE MYCOBACTERIUM TUBERCULOSIS

By James Reid, M.R.C.V.S., D.V.S.M.,
Veterinary Laboratory, Ministry of Agriculture and Fisheries.

Two instances of infection of bovines by human type of tubercle bacillus have been recorded in Great Britain.

Griffiths (1913) reported the finding of human-type infection in the mesenteric gland of a calf which had received milk from an animal vaccinated with human tubercle bacilli. The same writer (1936) proved infection in a calf which had reacted to the tuberculin test and in which lung lesions were subsequently found to be due to human type infection. This calf had had contact with a phthisical person.

The Scandinavian workers, Stenius (1933), Hindersson (1933) and Bang and Jepsen (1936), have recorded cases of tuberculin reactions in cattle due to sensitisation with human type tubercle bacillus. Stenius suggests that when no lesions are found at *post-mortem* examination in reacting cattle in Scandinavia, it may be either a case of bovine type infection in its incubation period or human type infection.

Since the Attested Herds Scheme was introduced by the Ministry of Agriculture, it has been the practice to purchase for special investigation a number of animals which give anomalous results in official herd tests in the field. In April, 1936, at a field test a 15 months old Guernsey heifer gave the following reactions in the double intradermal neck. caudal fold, and vulva tests, which at that time were carried out simultaneously.

Tuberculin used.	*Double Intradermal. Skin Measurement M.M.*			*Description of Reaction.*		*Caudal Fold. Description of Reaction.*	*Vulva. Description of Reaction.*
	Before Injection.	48 *hours.*	72 *hours.*	at 48 *hours.*	at 72 *hours.*		
Cambridge Synthetic Tuberculin	6	8	18	S.P.	P. D.O.	Diffuse thicking to three times normal	Negative

S.P. = Slightly painful. P. = Painful. D.O. = Diffuse œdema.

Ten days later, when the animal was tested at Weybridge with bovine and avian tuberculins, the following results were obtained:—

Site of Inoculation.	Tuberculin used.	Initial Skin Measurement at 1st Inoculation.	Observations at 48th hour and 2nd Inoculations in Neck Sites.	Final Observations at 72nd hour.
Right Side Neck ...	Bovine Tuberculin	8	16	20 Oedematous, hot and painful
„ „ Caudal fold	„		+	+
„ „ Vulva ...	„		+	+
Left „ Neck ...	Avian Tuberculin	8	12	14 Circumscribed and painful
„ „ Caudal fold	„		—	—
„ „ Vulva ...	„		—	—

The heifer was slaughtered and subjected to a careful *post-mortem* examination. No macroscopic lesions of tuberculosis could be detected in any organ or lymphatic gland. Suspensions were prepared from the lymph glands grouped as follows:—

(*a*) Prescapular, Popliteal and Precrural Glands.
(*b*) Parotid, Pharyngeal and Submaxillary Glands.
(*c*) Mediastinal and Bronchial Glands.
(*d*) Parotid, Iliac, Mesenteric and Supramammary Glands.

Four guinea-pigs, two rabbits and two tuberculin-tested fowls were inoculated with material from each group.

In the three groups (*a*), (*c*) and (*d*) the small animals and fowls were healthy when killed for *post-mortem* examination, at the end of two months. All were negative for tuberculosis. The fowls before being killed were tested and gave negative reactions.

In group (*b*) one guinea-pig and one fowl died from causes other than tuberculosis. One guinea-pig died from acute generalised tuberculosis at the seventh week following inoculation and the remaining two guinea-pigs in this group revealed acute generalised lesions of tuberculosis when autopsied at the eighth week. One rabbit inoculated intravenously showed generalised tuberculosis, while the second rabbit inoculated subcutaneously had a localised lesion at the site of inoculation.

The two fowls in this group (*b*) remained healthy, gave negative reactions when tested and were negative for tuberculosis at autopsy.

Material from the positive guinea-pigs, *i.e.*, those inoculated with material from parotid, pharyngeal and submaxillary glands, was seeded on to potato and egg media with and without the addition of glycerin. Growth on ordinary media was scanty, while the addition of glycerin gave luxurious growth on both potato and egg media. The appearance of the growth was suggestive of human type tubercle bacilli.

The following series of small animals was inoculated with the stated amounts of weighed dry culture.

(1) Two rabbits received 0·1 milligramme by intravenous inoculation.
(2) Two rabbits received 10·0 milligrammes by subcutaneous inoculation.
(3) Two rabbits received 0·1 milligramme by subcutaneous inoculation.
(4) Two rabbits received 1·0 milligramme by intraperitoneal inoculation.

In addition eight guinea-pigs received doses of bacilli varying from 1 to 0·1 milligramme by subcutaneous inoculation.

All the above animals, without exception, survived for a period of two months following inoculation.

One guinea-pig died on the 61st day following inoculation. *Post-mortem* examination revealed generalised tuberculosis. The remaining seven guinea-pigs were autopsied after the ninth week and each showed generalised tuberculosis.

All rabbits remained in good condition until examined at the end of nine weeks, when *post-mortem* findings were as follows:—

Rabbits Group (1).—These two rabbits showed a very few small grey caseous nodules in the lungs in which acid-alcohol-fast bacilli were sparse.

Groups (2) *and* (3).—The four rabbits in these groups revealed only lesions at the site of inoculation varying in size from ¼ to ¾ inch in diameter.

Group (4).—The two rabbits inoculated intraperitoneally both had small pea-sized tubercles in the intestinal wall.

It will be observed that the rabbits included in Groups (2), (3) and (4) revealed purely localised lesions and those in Group (1) showed lesions of a definitely non-progressive character.

Two young goats were inoculated subcutaneously, one receiving 50 milligrammes and the other 25 milligrammes of dried weighed bacilli. Both goats remained in good condition and were slaughtered after 14 weeks from time of inoculation. In both goats *post-mortem* examination revealed only localised lesions at the sites of inoculation.

Two young calves received subcutaneous inoculations of 10 milligrammes and 50 milligrammes respectively of dried weighed bacilli. Both remained healthy and gained weight until slaughtered eleven weeks after inoculation. On *post-mortem* examination both showed non-progressive localised lesions. The results of the cultural and quantitative biological tests are in accordance with the reactions associated by Griffiths (1930) with the human type of *mycobacterium tuberculosis*.

Attempts were made to trace contact between this heifer and a case of open human tuberculosis. The animal had been purchased

by the owner five months previous to the field test at which she reacted. Health reports of attendants on the last owner's premises were satisfactory, but information concerning the animal's previous attendants could not be obtained.

I wish to record my thanks to Mr. C. G. Cosson for his assistance.

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BOVINE TUBERCULOSIS IN THE TROPICS, WITH SPECIAL REFERENCE TO UGANDA

Part I.

By J. Carmichael, D.Sc., M.R.C.V.S.,
Veterinary Research Laboratory, Entebbe, Uganda

Tuberculosis as a disease in cattle has not, up to the present time, attracted much attention in tropical Africa and amongst the indigenous cattle in the tropics as a whole it is considered to be infrequent.

Veterinary activities have been concerned with the more dramatic and rapidly fatal diseases of domestic animals, and tuberculosis has been overshadowed by such epizootics as rinderpest, pleuro-pneumonia, East Coast fever, anthrax, etc., which from time immemorial have ravaged the flocks and herds in tropical countries.

In recent years, owing to closer administration and the success obtained in dealing with these major epizootic diseases, more attention has been devoted to tuberculosis. It has, however, only recently been recognised as existing at all in many countries.

The general apathy of the native cattle-owners to all diseases, especially those which are not responsible for heavy immediate losses, renders detection of tuberculosis difficult.

Meat inspection in tropical Africa is a very recent innovation and by no means a universal custom, and the vast majority of meat consumed by natives is uninspected even at the present time.

In tropical Africa and Asia almost all the indigenous cattle are of the Zebu type (*Bos indicus*), although, as will be pointed out later, there are areas which are characterised by non-Zebu animals.

India.

In India, up till quite recently, tuberculosis has been considered a rare disease in the indigenous cattle. Mitter (1910) reported four cases of bovine tuberculosis in Calcutta. Three were in Zebu cattle and one in a buffalo. He records that during the previous 12 years there 45 fatal cases of tuberculosis in cattle at the hospital of the Bengal Veterinary College. Almost without exception these occurred in Zebu cows.

Liston and Sopakar (1917) state: "According to the opinion of a large number of observers bovine tuberculosis is of rare occurrence in India." This condition of affairs has been attributed to the open-air existence of Indian cattle as compared with cattle kept in Europe, a supposition which is frequently met with in the literature on bovine tuberculosis in the tropics and which seems to be a popular explanation for the comparative rarity of the disease.

As will be pointed out later, this would certainly not apply in Uganda or in fact in most parts of tropical Africa, since the vast majority of cattle are never housed.

The above authors (Liston and Sopakar, 1917) carried out experiments with a view to ascertaining whether the local Indian breeds are more resistant to tuberculosis than their European counterpart.

Twelve buffalo and twelve cow-calves from four to eight months old were inoculated subcutaneously in front of the shoulder with a virulent strain of *Mycobacterium tuberculosis* obtained from Dr. Eastwood which was used in the experiments of the English Royal Commission on Tuberculosis.

The doses used were 50 mg. and 10 mg. In their protocols the authors record the changes occurring at the seat of inoculation, the condition, body weight and daily temperatures throughout the experiment. The results of the *post-mortem* examinations are also given and elaborate figures are drawn to illustrate the distribution of the lesions in Indian calves as compared with those described in the case of English calves experimented on under similar conditions, as detailed in the reports of the English Royal Commission on Tuberculosis.

The results of the experiment indicated that the comparative infrequency of the disease among cattle in India is due to a natural resistance rather than to any method of housing or keeping cattle in India as compared with England.

The question of the comparative resistance to tuberculosis exhibited by Zebu cattle in Africa will be considered in detail in a subsequent paper.

Taylor (1918) carried out an enquiry as to the prevalence of bovine tuberculosis in the Punjab from August, 1915, to March, 1916, during which period 3,276 animals were admitted for slaughter at Ferozepore. Examination of all animals was performed prior to slaughter and 575 or 17·8 per cent. were rejected as not likely to be fit for human consumption on account of innutrition, disease or old age. It is probable that a good many of these cast animals were suffering from chronic tuberculosis. Of the remaining selected animals 95 or 3·5 per cent. proved to be tuberculous. In 60 of these cases tubercle bacilli were demonstrable in the lesions on microscopic examination. The lesions were mainly localised, and the bronchial and mediastinal glands were most commonly affected.

During the year 1916-1917 (April 1st to March 31st), 4,610 animals were brought for slaughter. Of these, 821 were rejected as unfit, and of the remaining animals 125 or 3·3 per cent. were found infected.

The question of bovine tuberculosis was discussed at the first meeting of veterinary officers in India (1919) held at Lahore. Olver pointed out that no real scientific enquiry had yet been carried out in India upon the incidence of tuberculosis in cattle. At Cawnpore 3 per cent. of the cattle at the slaughter-houses were found to be infected. Lt.-Colonel A. Smith stated that about one case a year during the past 23 years had occurred in Calcutta. Major K. Hewlett said that no evidence of tuberculosis had been found in

the Bombay slaughter-houses. Other speakers pointed out that tuberculosis was absent or very infrequent in Madras, Assam and Bihar and Orissa. G. Taylor referred to the large number of cases found in the slaughter-houses at Ferozepore and Simla, and gave his opinion that tuberculosis was fairly prevalent in Northern India. Sowerby (1923) reported a case of tuberculosis in a seven-year-old cow in the Bombay Presidency. Lesions were present in the lungs, liver and udder.

Liston and Sopakar (1924) isolated nine strains from various animals at the Bombay Zoological Gardens, eight of which resembled the bovine virus. One strain was indistinguishable from the human type—this from a Binturong. The others were from antelope and a llama. Naik (1932) reported a case of generalised tuberculosis in an Indian buffalo.

In Ceylon, 1934, there were over 40,000 bovine carcases inspected, of which only one was found to be tuberculous.

Tropical Africa.

As far as Africa is concerned the general impression is that the disease is rare among the indigenous cattle; thus, only accounts of sporadic cases are found recorded in the literature.

West Africa.

Most of the cases have been reported from the French possessions. Curasson (1919) reported that he had met cases of tuberculosis of the udder in native cattle at Dakar, the bacilli being of the human type. No bacteriological evidence supports this view.

In Dahomey, Curasson (1920) reports a tuberculosis morbidity rate of about 33 per cent. in a race of cattle known as Lagunes. These cattle appear to be an isolated breed and are said to be badly stalled. The report is interesting in view of the experience in Uganda with the Ankole cattle.

Teppaz (1922) records a case of tuberculosis in a Zebu animal at the slaughter-house at Dakar, the first example of bovine infection seen by him in many thousands of cattle he examined, both at Dakar and Saint Louis. He states that the bacilli were probably of human origin but produces no evidence for this unorthodox view.

Nainsouta (1922 and 1923) records two further cases of tuberculosis in Zebus at the Dakar abattoirs. Tubercular lesions ranging in size from a nut up to that of a man's fist were found in the lungs. The bronchial and mediastinal glands were enlarged and tuberculous.

Teppaz (1923) reported another case from Dakar, the fourth to be discovered. The animal was a Zebu and the lesions were confined to the lungs. A guinea-pig which was inoculated from this case died on the 26th day from generalised tuberculosis. A rabbit was also inoculated and apparently did not become infected.

Teppaz (1923) further records that tuberculosis, whilst not absent in French West Africa, is rare. In an abattoir killing 20 to 30 animals a day he only found six cases in three years.

Curasson (1928) amplifies the findings of Teppaz in French West Africa. He believes that tuberculosis occurs more frequently than has been suspected. In 1925 there were 26 cases found among 4,205 Zebus slaughtered at Bamako. In 1934, at the same place, out of 7,573 cattle slaughtered there were 49 cases of tuberculosis, of which 22 were generalised. At Dakar five cases were discovered out of a total of 12,818 carcases inspected.

In the British colonies in West Africa tuberculosis in cattle is rare. Hall (1930) recorded the first case in Northern Nigeria. This animal died and on *post-mortem* examination proved to be an acute case of pulmonary tuberculosis showing *perlsucht* lesions on the pleuræ, numerous tubercles in the parenchyma of the lungs, and involvement of the bronchial and mediastinal glands. The organism was isolated in culture and proved to be a typical dysgonic bovine type. The disease has been reported on several occasions in S. Nigeria (1930).

In 1931 the cattle at the laboratory at Vom were tuberculin-tested. Seventeen reacted and of these seven showed lesions on *post-mortem*.

In 1934 six cases of tuberculosis were discovered at the slaughter-houses in N. Nigeria, whilst in 1936 the incidence among cattle slaughtered at Calabar abattoir "assumed serious proportions."

Out of 1,035 cattle slaughtered 158 or 15·26 per cent. showed lesions of tuberculosis. The Zebu cattle of the Fulani in N. Nigeria seem to offer resistance to the disease.

On the Gold Coast, bovine tuberculosis is uncommon. No cases were reported in the years 1930-37 inclusive.

In the Cameroons, Zeemann (1913) reported the absence of the disease in the indigenous cattle.

Manley (1929) carried out tuberculin tests on cattle owned by a German Company in the British Cameroons. The cattle were cross-bred Zebu-native or Algauer-native and pure-bred Algauer stock. Of the 56 animals tested 14 reacted, of which 10 were cross-bred cows. The disease was apparently introduced from Germany by the imported pure-bred stock.

In the Belgian Congo, Mouchet (1913) stated that bovine tuberculosis did not exist in the area of Leopoldville.

Egypt.

Tuberculosis exists in the cattle. Littlewood (1920) reported 3,524 cases detected almost entirely in the Cairo and Alexandria abattoirs, which was 5·21 per cent. of the animals slaughtered. Of 2,210 cattle belonging to the Domains administration which were tested with tuberculin 963 or 43·5 per cent. reacted.

Sudan.

Tuberculosis appears to be extremely rare. One case was reported in 1932 from the Bahr-el-Ghazal Province. The Director of Veterinary Services sums up the position as follows: "In consideration of the number of cattle slaughtered in controlled centres for food and of the fact that several hundred cattle are annually examined *post-mortem* in the veterinary laboratories at Khartoum and Malakal, the disease must be very uncommon." In the report of the Veterinary Services, Egypt, 1916 and 1917 (Littlewood), there is mention of one case of tuberculosis in a bull from the White Nile Province, the first case from that locality. Over the previous five years eight cases occurred and all emanated from Kordofan Province. These were in Sudanese cattle slaughtered in Egypt.

As far back as 1912 Archibald reported a suspected case of tuberculosis in a camel in the Sudan but no cultural tests or animal inoculations were performed.

Kenya.

Tuberculosis is rarely mentioned in the annual reports of the Veterinary Department. In 1933 two cases were diagnosed in Suk cattle. This is of interest as the Suk country borders on Karamoja in Uganda from where several cases have recently been discovered in the Kampala abattoir. Occasionally sporadic cases are reported from the Masai cattle.

Tanganyika Territory

In the annual report of the Veterinary Department, McCall (1929) records that only four cases of tuberculosis in Zebu cattle in the territory are on record. Further reports give the following information:—

1930—"Extremely rare."
1931—"Extremely few cases of this disease reported."
1932—Of 15,423 carcases inspected there were two cases which occurred at Iringa.
1933—There were 14,904 carcases inspected, of which nine were tuberculous.

In 1936, 42 cases were reported from the abattoirs in the southern highlands. No cases of generalised tuberculosis were reported and the disease in cattle is considered unimportant in most parts of Tanganyika.

Cornell (1934) tested with tuberculin 441 cattle (Zebu) belonging to the Wachagga tribe on the slopes of Kilimanjaro. There were eight reactors but no lesions were discovered in four of these which were slaughtered. Of 13 doubtful reactors one was slaughtered but no lesions were present. The Wachagga keep their cattle in their huts where they are hand-fed, and the incidence of tuberculosis both pulmonary and surgical is high among these people.

Hornby (1934) reviewed the position with regard to bovine tuberculosis in tropical Africa and Tanganyika Territory in particular, and points out that a proportion of the total cases reported are connected in some way with imported European stock. He states: "A reasonable view seems to be that native races of cattle, particularly the Zebu, are more resistant to tubercle infection than are cattle of European race, and that the more open-air life led by the majority of native cattle further contributes to a low incidence of tuberculosis." Before accepting such an hypothesis much more concrete evidence would appear to be desirable.

Nyasaland.

Tuberculosis in cattle seems to be rare. There were two cases reported in 1926, and one suspected case in 1934.

Northern Rhodesia.

The disease appears to be associated with imported cattle. Purchase (1929) found 90 reactors out of 444 cattle in an old-established herd in the Fort Jameson district. The animals were chiefly Hereford-native and Shorthorn-native crosses. The highest number of reactors occurred among the young stock, some 26 per cent.

More recently (1937) it is stated that tuberculous infection in cattle is rather more widely disseminated than was at one time thought.

In Southern Rhodesia and the Union of South Africa there is so much imported European stock that it is almost impossible to get accurate figures for the purely native breeds.

Bovine Tuberculosis in Uganda.

The cattle in Uganda, which number approximately two million head, are divisible into three groups. Firstly, the Zebu group (Fig. 1) which is the predominating race of cattle in equatorial Africa. Their numbers in Uganda easily surpass those of the remaining two groups. They occupy all the Eastern and most of the Northern Provinces.

The second group consists of the Ankole cattle (Fig. 2). These animals are characterised by enormous horns. They have no hump and are much longer in the leg than the Zebu animals. They exist as a highly characteristic group of cattle amidst the usual tropical African short-horned, humped animals. Their origin is unknown, but the generally accepted theory is that they came from the Galla country of Abyssinia and were brought south and west with their Hamitic owners—the Bahima. The Ankole cattle occupy Ankole proper and the western part of Buganda Province, besides small areas in Tanganyika and the Belgian Congo.

FIG. 1. Zebu Cattle.

FIG. 2. Ankole Cattle.

There is situated between the Ankole and the Zebu cattle a third group of cattle which is intermediate between the long-horned Hamitic type and the short-horned Zebu. These cattle occupy most of Buganda Province, most of Toro and Bunyoro, and also Kigezi in the west.

History and Incidence.

Almost the whole of the bovine tuberculosis exists in the Ankole cattle and the incidence, as will be illustrated later, is extremely high. In the Zebu and intermediate types tuberculosis, whilst not rare, is certainly infrequent.

The first record of bovine tuberculosis in Uganda was in 1917, when lesions were discovered in some Ankole cattle sent to the Kabete Laboratory in Kenya for the manufacture of anti-rinderpest serum.

In 1918, the late Mr. E. Hutchins, M.R.C.V.S., the Chief Veterinary Officer, in a pamphlet on "Animal Diseases," under the heading "Tuberculosis," records that: "Only one case has hitherto been met with amongst cattle in Uganda."

In the annual reports of the Veterinary Department, 1919 to 1928 inclusive, tuberculosis is not mentioned as occurring in the Protectorate. In 1929, seven cases of tuberculosis, all in Ankole cattle, are reported and the following observation made: "The number of cases exceeds those reported in previous years but this cannot be interpreted as any proof of an increase in the incidence of the disease. Circumstances have allowed a greater number of carcases of Ankole cattle to be examined."

In 1930, 13 cases were recorded. The year 1931 showed the first marked increase in the number of cases discovered and 48, of which 21 were generalised, were discovered in 277 carcases inspected at Mbarara.

Further investigations were carried out in 1932, when no less than 140 cases were observed in 1,654 Ankole cattle, and three cases in 2,600 Zebu carcases. It should be explained that in that year, owing to successful disease control, the trade in slaughter cattle, especially from the Eastern Province (Zebu area), greatly increased.

In 1933 the figures were as follows:—

Ankole cattle: 2,874 carcases examined, 359 or 12·5 per cent. showed tuberculous lesions.

Zebu cattle: 4,458 carcases examined, 31 or 0·7 per cent. showed tuberculous lesions.

During that year the opportunity was taken to examine 131 Ankole cattle which had died of rinderpest. Of these 51·1 per cent. showed visible tuberculous lesions.

In 1934, at one market near Kampala, tuberculosis was observed in 50 out of 2,067 Zebu animals or 2·41 per cent., whilst out of 685 Ankole animals no less than 233 or 34 per cent. contained lesions.

The *post-mortem* statistics, including 1935, are tabulated below.

Table Showing Incidence of Tuberculosis in Uganda Cattle as seen *Post-mortem*.

Year.	*No. of Carcases Examined.*	*Types of Cattle.*	*No. of Cases of T.B.*	*Percentage Incidence.*
1931	277	Ankole	48	17·3
1932	1,654	Ankole	140	8·4
	2,600	Zebu	3	0·1
1933	2,874	Ankole	359	12·5
	4,458	Zebu	31	0·7
1934	685	Ankole	233	34·0
	6,215	Zebu	143	2·3
1935	464	Ankole	192	41·4
	5,858	Zebu	29	0·5
1936	231	Ankole	77	33·3
	7,848	Zebu	49	0·6

At one market near Kampala, in 1935, the lesions of tuberculosis were found to be according to the table below.

Table Showing Distribution of Tuberculous Lesions.

No. of Cattle.	*Type of Cattle.*	*Total No. Tuberculous.*	*Distribution of Lesions.*		
			Thoracic Cavity.	*Abdominal Cavity.*	*Generalised Infection.*
464	Ankole	192 (41·4 per cent.)	96 (50 per cent.)	75 (39 per cent.)	21 (11 per cent.)
1,334	Zebu	13 (0·97 per cent.)	12 (92 per cent.)	1 (8 per cent.)	— —

Incidence of Tuberculosis in Cattle in Uganda as Demonstrated by the Tuberculin Test.

All the tests were carried out by the double intradermal method and the technique employed was that advocated by the Medical Research Council (1925).

Owing to the frequency of conjunctivitis and other inflammatory conditions of the eye the ophthalmic test is unreliable in Uganda, whilst the normal fluctuations in the temperatures of healthy cattle in the tropics nullifies the subcutaneous method.

None of the cattle tested were housed, and all lived entirely in the open air.

General Discussion and Consideration of the Results.

A consideration of the slaughter-house returns and the tuberculin tests reveals a strikingly high incidence of tuberculosis in the Ankole stock, and a comparatively low morbidity rate in the Zebu and intermediate types.

Tanganyika Territory. Annual Reports of the Department of Veterinary Science and Animal Husbandry, 1929-1936, inclusive. Govt. Press, Dar-es-Salaam.

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REVIEW

Pathological Histology, By Robertson F. Ogilvie, M.D., F.R.C.P., (Edin.). Lecturer on Pathology, University of Edinburgh. Foreword by A. Murray Drennan, M.D., Professor of Pathology, University of Edinburgh. Pages 331. With 220 illustrations. E. and S. Livingstone, Publishers, Edinburgh. Price 27s. 6d. net (postage, 7d.).

In the preface it is stated that this work is designed to act as a companion to a text-book of pathology and to meet the need of the student in the class of morbid histology and of the graduate seeking more special knowledge in pathological processes. It is based on a series of lectures on pathological histology delivered at Edinburgh and deals with the tissue changes produced in the diseases most commonly encountered in Great Britain.

A large proportion of the 220 coloured microphotographs present to the student's eye stained sections, films, etc., from diseased organs or tissues. It may be said, without detriment to the text, that the number and excellence of these illustrations form the leading feature of the work, and will specially increase its value to the student in a practical class who is endeavouring to follow the teacher's account of the object under the microscope.

Like nearly all modern text books on the pathology of man the present work leaves the subject of bacteriology untouched except with regard to the visible effects produced by bacteria, but that is a custom that can be defended on the ground that it is required in order to make possible the inclusion of the cause as well as the consequence of disease within the time allowed in in the medical curriculum.

The present volume is well designed to help students to acquire the knowledge of bacteriology required to enable them to pass the examination in that subject necessary for admission to the profession of medicine.

It certainly deserves to rank high among works of the same kind previously published in the English language.

The Publishers' share of the work also deserves praise. The paper, type and binding are good.

Printed in Great Britain by H. R. Grubb, Ltd., Croydon.

TABLE SHOWING RESULTS OF TUBERCULIN TESTS.

No. Tested.	*Sex.*	*No. of Cattle.*	*No. Positive.*	*Remarks and Observations.*
5	Bulls	Ankole	1	Killed and found tuberculous
2	„	Intermediate (Kigezi)	—	—
19	„	„	3	All 3 killed and found tuberculous
4	„	Ankole	2	1 killed and found tuberculous
90	Mixed	Intermediate	7	3 „ „
42	„	Ankole	19	No *post-mortem* carried out
44	„	„	45 (per cent) 9 (20 per cent.)	On these two herds 14 *post-mortems* were carried out on 17 reactors and lesions were found in 13
50	„	„	8 (16 per cent.)	
15	„	Mixed Ankole and Intermediate	3	No *post-mortem carried out*
9	Young Bulls	Ankole	1	
6	Bulls	Zebu	0	„ „
1	Old Cow	Ankole	1	„ „ Killed and found tuberculous
4	Bulls	„	2	No *post-mortem* carried out
4	„	Zebu	0	„ „
6	Young Bulls	Ankole	5	„ „
205	Mixed	„	157 (76·6 per cent.)	„ „
121	„	Intermediate (Kigezi)	6 (5·2 per cent.)	„ „
12	Bullocks	Zebu	0	All eventually examined *post-mortem* and no lesions present
	(N.B.—These cattle were housed with tuberculous natives in Bugishu)			
158	Mixed	Zebu	1 (0·6 per cent.)	No *post-mortem* carried out

Except in the case of a few individual tribes native cattle are never housed. It is, however, the almost universal custom to nurse a sick animal in the hut of the owner, whilst the calves usually share the human dwelling places.

In Ankole the cattle roam over short grass down-like country and at night they are put into a kraal or round enclosure of thorn bushes.

The rainfall in Ankole is in the region of 30 inches per annum with two dry seasons of approximately three months each. It is the custom to burn off the grass at the end of each dry period in order to provide fresh grazing when the rains commence.

In order to ward off biting flies and mosquitoes, a smudge fire of dried cow dung is lit just before sundown, and the cattle pack themselves head to head round these fires apparently relishing the smoke.

The close contact and the coughing due to the smoke may well facilitate the spread of tuberculous infection from one beast to another.

The disease is well known by the Bahima cattle owners. These people are a pastoral tribe of Hamitic strain whose staple diet is the milk and blood of their cattle. In the Luhima language the disease is called "Ruhosho" and according to tribal legend it has always been present both in the cattle and in man. There seems to be no information as to when and where the disease originated.

No European-bred stock has ever crossed the tribal boundary. In fact European cattle, to all intents and purposes, are non-existent in Uganda except on one or two Government Stock Farms, as the presence of East Coast fever and other tick-borne diseases prevents any imported cattle from surviving for more than a few weeks, except under special conditions.

The interesting feature is the high incidence of tuberculosis in this one isolated breed of Ankole cattle in Central Africa.

The whole history of the Bahima people and their cattle is uncertain and bound up with complicated anthropological questions and it may well be possible that tuberculosis came with these people in their legendary trek from the Hamitic area of North-East Africa (Driberg, 1930).

On the evidence accumulated one naturally assumes that Ankole cattle appear to be more susceptible to tuberculosis infection than the truly indigenous Zebu cattle of Central and East Africa. Experiments designed to investigate this question have been carried out and will be discussed in the second part of this paper.

Observations on the Disease, Nature of Lesions, Native Treatment, etc.

From the figures given it would appear that bovine tuberculosis has increased considerably in Uganda during recent years. Owing to the lack of any control measures and the primitive methods of husbandry, there can be no doubt that the disease is on the increase for the simple reason that there is nothing to prevent it.

The figures given in this paper, however, cannot be considered as significant of a serious increase in the incidence of the disease as the general facilities for inspection, and the number of cattle slaughtered, have increased enormously during the past ten years. In a young country the usual course of development is bound to reveal the situation as time goes on.

It is uncommon to find generalised cases of bovine tuberculosis in Uganda, though it is probable that these occur at times in the native kraals and are not reported. In the majority of cases the lesions are found in the mediastinal and bronchial glands and lungs with no evidence of intestinal infection. Tuberculous foci are, however, not rare in the abdominal cavity.

It would seem that the main portal of entry is through the respiratory tract and the custom already referred to of crowding round the smudge fire in the kraal would facilitate this mode of infection. It is evident from the localised infection in the glands that the Uganda cattle offer a considerable resistance to the disease and are not, in Cobbet's words, "virgin soil" for infection with tuberculosis.

There is no doubt that tuberculosis has been recognised by the natives of Ankole for many years. It is extremely rare to find cases showing the classical grape-like clusters of tubercles or *perlsucht* lesions on the pleuræ and only one case was discovered out of several hundred tuberculous cattle examined in Kampala abattoir in 1934, whilst tuberculous mastitis is not common.

The suggestion that there is a relationship between the incidence of pleural and peritoneal lesions and those in the mammary gland is perhaps worthy of investigation as little is known of the precise lymphatic drainage of these parts.

Although tuberculosis is known to the Bahima people of Ankole, the cattle-owning natives in the Bantu and Nilotic areas of Uganda do not appear to recognise the condition.

One of the commonest ways of treating the disease in Ankole is by branding the chest-wall with hot irons. Various vegetable infusions are also prescribed by the tribal "cattle doctors" in the hope of effecting a cure of the disease.

Strains of Mycobacterium Tuberculosis Occurring in Cattle in Uganda.

Until these investigations were initiated little or no work had been carried out in connection with the type of organism causing bovine tuberculosis in Eastern Africa.

In West Africa, however, various French workers have on occasion incriminated the human type of organism but in the absence of bacteriological proof these can be discounted.

At this laboratory 39 strains of the tubercle bacillus have been isolated from both Ankole and Zebu cattle and they have all proved to be typical dysgonic bovine strains of normal virulence.

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